

**The relationship between peak lean tissue velocity  
and peak bone mineral content velocity during the  
adolescent growth spurt.**

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**By**

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## Abstract

It has been theorized that muscles generate more force on bone than body weight alone and therefore it is likely that muscle contraction drives and sustains bone adaptation (Frost 1999). **Purpose:** To investigate the relationship between the timing and tempo of peak growth velocities of lean tissue (LT) and bone mineral content (BMC) in boys and girls at three sites using data derived from individual growth curves. **Methods:** 72 boys and 70 girls were fitted with growth curves that had a distinguishable peak. Height and weight were measured for each participant and tissue assessment was performed annually using DXA. Factorial ANOVAs were completed to analyse data for differences in age, while forward regression analyses was used between LT and BMC. **Results:** The peak growth velocity for lean occurred significantly ( $P < 0.05$ ) earlier than the peak growth velocity for bone at all locations except the legs. There was a difference ( $P < 0.001$ ) between genders in the age of peak for both lean tissue and bone tissue at all locations with females peak growth occurring before that of males. When aligned by PHV a significant difference ( $P < 0.05$ ) in the timing of PBMCV was found between the arms and the legs with the peak in bone growth in the legs occurring significantly before peak bone growth in the arms. PLTV was independently associated with PBMCV at the arms ( $r^2 = .71$ ,  $p < 0.001$ ), legs ( $r^2 = .53$ ,  $p < 0.001$ ) and trunk ( $r^2 = .52$ ,  $p < 0.001$ ). **Conclusion:** In conclusion, LT growth precedes BMC growth and after controlling for gender, size and maturity the magnitude of LT growth is associated with BMC growth. The findings of this study are in support the Muscle-bone Unit (Frost and Schoenau, 2000), which theorises

that localised muscle action is a driving force for bone growth. Future studies are needed to analyse bone strength as it relates to local muscle strength and usage while controlling for confounding variables.

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## **Dedication**

This thesis is dedicated to my family, who has made so much possible.

They are the ones who first taught me how important a healthy lifestyle is by promoting physical activity and proper nutrition. Summers at the lake, swimming lessons, days at the beach and winters in the rink were just the beginning of pursuing an education and later launching a career in kinesiology. Not to mention all the time and effort that went into sport camps, road trips, and providing new equipment. This balanced with learning at an early age what “home cooking” was and developing the ability to appreciate the goodness of healthy foods.

Their encouragement and assistance has been the motivation to succeed  
in all aspects of life.

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## **List of Abbreviations**

ANOVA	-Analysis of Variance
BMC	-Bone Mineral Content
BMCcort	-Cortical Bone Mineral Content
BMD	-Bone Mineral Density
BMI	-Body Mass Index
BMOp	-Bone Marrow Osteoprogenitor
BMU	-Bone Modeling Unit
CAHPER	-Canadian Association for Health, Physical Education & Recreation
DONALD	-Dortmund Nutritional and Anthropometric Longitudinal Study
DXA	-Dual X-ray Absorbtiometry
IGF-1	-Insulin-like Growth Factor 1
MRI	-Magnetic Resonance Imaging
NHANES	-National Health And Nutrition Examination Survey
PLTV	-Peak Lean Tissue Velocity
PBM	-Peak Bone Mass
PBMAS	-Pediatric Bone Mineral Accrual Study
PBMCV	-Peak Bone Mineral Content Velocity
PHV	-Peak Height Velocity
PMSV	-Peak Muscle Strength Velocity
PQCT	-Peripheral Qualitative Computed Topomography
QUS	-Qualitative Ultra-Sound
SGDS	-Saskatchewan Growth and Development Study

TBC	-Total Body Calcium
TBK	-Total Body Potassium

## 1. Introduction

It is well documented that bone mass declines throughout adult life; thus most of the intervention strategies in adults and older adults are directed at reducing the rate of bone loss. In theory, the less bone that is lost with aging, the lower the risk of osteoporosis in later life. However, it also is thought that an equally, if not more important factor in affecting adult bone health, is the maximum amount of bone mass (or peak bone mass, PBM) that is accrued by early adulthood (Bailey et al. 1996). That is, optimizing the attainment of PBM during childhood and adolescence and the resulting increase in bone strength may reduce the risk of osteoporosis in later life.

Bone mineral accrual increases progressively through childhood and then accelerates during adolescence. The skeletal mass achieved at the time of peak mineral accrual determines over half the variability in skeletal mass after 65 years of age, and accounts for 80% of bone strength with the remainder being accounted for by geometry (Pettersson et al. 2000). During adolescence over half the variability in bone mass is accounted for. By the age of 18 at least 90% of peak bone mass is attained with 25% being acquired during the two year period surrounding peak height velocity (PHV) (Bailey et al. 1997).

Bone adapts to increased demand strain by either increasing its mass, altering its geometry or by improving its architecture to accommodate the increases in strain (Ferretti et al. 2002). In 1917, as cited in Frost (2001), Koch stated “the largest voluntary bone loads and bone strains come from muscles, not body weight as previously thought.” More recently it has been theorized that

muscles generate more force on bone than body weight alone and therefore it is likely that muscle contraction drives and sustains bone adaptation (Frost 1999). Bone adapts to the strains placed on it by the forces generated by muscles. Muscles action works against the resistance of body weight multiplied by the inefficient lever arms against which muscles work. The association between muscle forces on bone has been referred to as the muscle-bone unit (Frost and Schoenau, 2000). If the action of muscle forces on bone affects bone mineral accrual then this relationship should be most evident during the adolescent growth spurt when the magnitude of tissue growth is at its maximum. Changes in muscle strength should therefore precede a corresponding increase in bone strength. Using data from the University of Saskatchewan Paediatric Bone Mineral Accrual Study (PBMAS), Rauch et al. (2004) found that the peak lean body mass (a surrogate for muscle strength) accrual preceded peak bone mineral content (a surrogate for bone strength) accretion. They also found that the magnitude for lean body mass growth was independently associated with the magnitude of bone mineral content accrual during growth. These results support the theory that muscle action stimulates increases in bone strength.

Rauch's study applied data using group means to derive the growth curves; thus, they were not able to determine differences among individuals. A minimal amount of information can be obtained from cross-sectional data, however information gathered from longitudinal studies provides multiple reference points that allow change and rate of change over time to be analysed. Although, when data is pooled together in a cross-sectional analysis of the mean

of longitudinal data also does not account for individual variation in growth. Only the average change in growth with time can be analysed. When looking at the whole picture, longitudinal analysis of the individuals is required when investigating individual changes through various growth phases. There are currently no studies that have used individual growth curves to determine the timing and magnitude of muscle growth and its association with bone mineral accrual during adolescence. Longitudinal studies are essential to provide insight not only to mean data but also to allow for the examination of outliers in the data that might not follow the mean pattern of growth. With mean data there is a regression to the mean reducing the variability of subject change over the growing years. The purpose of this study was to explore the timing and tempo and/or magnitude of growth for both muscle and bone during the adolescent growth spurt for the legs, arms and trunk. A unique characteristic of this study is that it utilises data derived from individual growth curves from the Saskatchewan Paediatric Bone Mineral Accrual Study.

## **2. Review of the Literature**

Comprehension of basic bone physiology and the factors that affect changes to bone are required to understand the relationship between bone and muscle development throughout growth. Thus, this chapter includes preliminary sections on bone physiology and the effects of genetics, nutrition and physical activity on bone. The chapter then includes a review of the literature related to the effects of muscular activity on bone mineral accrual; including sections on Mechanostat Theory, bone and muscle growth and the relationship between muscle and bone. The final section focuses on bone and muscle growth and development during adolescence, including studies that have investigated the relationship of mechanical loading by muscle action on bone.

### **2.1. Bone physiology**

For mechanical influence to control biological activity, a mechanism must convert a mechanical signal to a physiological response. Mechanotransduction (Turner & Pavalko, 1998) is the physiological process by which bone adapts to the imposed demands. The mechanical force is perceived by sensor cells and converted to a biological signal. This biological signal then stimulates the effector cells that will form (osteoblasts) or remove (osteoclasts) bone, which produces the appropriate tissue-level response. The appropriate tissue response would then make the necessary adjustments to remove the signal halting the response.

Bone growth is a function of both modelling and remodelling activity. Modelling is organised bone cell activity that allows for strategic bone growth. Modelling is restricted to the growing years during early life prior to attaining



skeletal maturity. Remodelling allows for the renovating of bone by activating both osteoblastic and osteoclastic activity. Unlike the modeling drifts, bone remodelling turns bone over in small packets or BMUs (bone modeling units) after skeletal maturity has been attained. During adulthood there is a decrease in bone mass after skeletal maturity is reached because remodelling has a ratio for mineral accrual and resorption of approximately 19 to 20. During adolescence, the addition of new bone is the dominant function, whereas during adulthood remodelling is dominant and the removal of bone prevails. (Khan et Al. 2001)

Bone strength is a function of bone mass, geometry and architecture. An increase in bone mass alone would produce bone that is not functionally optimal. Increases in mass can be seen as disadvantageous because the individual must expend more energy for the purposes of movement and locomotion (Seeman 2001). For this reason, increases in mass alone are not sufficient and must be accompanied by improvements in bone architecture and geometry. Cortical bone makes up the dense outer layer of bone (75-80% of bone mass), while trabecular bone makes up an inner meshwork lining of thin trabeculae (20-25% of bone mass) that permits space for bone marrow, blood vessels and connective tissue throughout the central medullary cavity (Bailey et al. 1996). Circumferential growth by expanding the cortical layer allows for the greatest increases in bone strength. As a bone increases its periosteal (the outer surface of a bone) mass the endosteal (the inner lining of a bone surrounding the medullary cavity) bone is resorbed allowing for increases in the bones cross-sectional diameter; this action causes a moving of the mass further from the bones longitudinal axis with

a subsequent net increase in bone strength. With circumferential expansion, more bone must be laid down to accommodate the increases in bone size and increase bone strength. Therefore, growth builds a larger bone, not a denser bone. The final component of bone strength, bone architecture, is determined by the arrangement of minerals in the bone matrix.

Hormonal actions during growth directly affect muscle and bone mineral accrual. Testosterone (Leder et al. 2003), estrogen (Leder et al. 2003), insulin-like growth factor 1 (IGF-1) (Tirapegui, 1999, Murras et al. 2000, Snow et al. 2000, Woods et al. 2000, Nindl et al. 2001), other growth hormones (Murras et al. 2000, Godfrey et al. 2003), leptin (Sun et al. 2003) and parathyroid hormone (Zitterman et al. 2002) have been shown to be significantly involved in the growth of muscle and bone. These hormones stimulate both bone and muscle growth by facilitating growth through various physiological mediators. Hormone levels alone are not sufficient to elicit bone mineral accrual; that is, an external stimulus is required on the bone to initiate hormone activity leading to mineral accrual. Consequently, the possibility of a genetic relationship between muscle and bone due to hormonal influences does not necessarily disprove a muscle-bone relationship. The body could have developed so that adaptations would be driven by a shared system; therefore, changes in one would be met by subsequent changes in the other, in either a positive or a negative direction. For example, skeletal unloading has also shown an inverse relationship in rats, where the unloading induces resistance to IGF-1 administration in vivo in bone tissue and BMOp (bone marrow osteoprogenitor) cells (Sakata et al. 2003).

Therefore, IGF-1 stimulates proliferation only in the cells of loaded bones.

### **2.1.1. Mechanostat**

There is an observed relationship between a healthy individual's bone mass and typical mechanical usage. The mechanical force is perceived by sensor cells and converted to a biological signal. This biological signal then stimulates the effector cells that will form or remove bone, producing the appropriate tissue-level response. The appropriate tissue response would then make the necessary adjustments to remove the signal, thus halting the response.

Frost (1987) proposed the Mechanostat theory, to explain bone adaptation. Frost suggested that the mechanostat functions in three basic windows: A disuse window that prompts bone resorption, (below 300 microstrain); a physiological window that facilitates maintenance (300-1500 microstrain) and finally an overuse window that would stimulate the formation of new bone at a microstrain above 1500 (Frost. 1989). The Mechanostat theory postulates that bone adapts in a manner comparable to a household thermostat. A negative feedback loop controls activity with respect to a given set point. In a household, when the temperature becomes too cool the thermostat senses the change in temperature and turns on the furnace to heat the house to the desired set point. In bone, the set point is a given mechanical strain on the bone. Although there are set points for the thresholds, these set points are variable and adjust according to individual conditions experienced by the body. For a given individual, the set point could be higher or lower and it is directly related to the status of the effectors of mechanical bone density. These effectors could be

mechanical or hormonal. or could result from disease. When the body undergoes a drastic change, the set points are readjusted accordingly. For example, calcitonin and fluoride have anabolic effects by inhibiting bone resorption (Turner, 1991) while the absence of estrogens, such as during post-menopause, can lead to bone resorption and osteoporosis (Sugiyama, 2002). These factors are considered non-mechanical effects on bone metabolism and either optimize or inhibit bone structural adaptations (Frost, 1998).

## **2.2. Factors affecting bone growth**

Bone mass accumulation involves interrelated actions of genetic, endocrine, mechanical and nutritional factors. Bone growth is largely determined by genetics and is gender specific (Slemenda et al. 1994, Schoenau et al. 2000, 2002, Illiano-Burns et al. 2001). Although, according to Chestnut (1991), genetics may regulate bone formation but not bone resorption, and environmental as well as genetic interactions contribute to observed bone mass at later ages. These modifiable factors may determine whether or not an individual is able to achieve their genetic potential. Other non-modifiable growth factors such as ethnicity, (Wang et al. 1999, Bachrach et al. 1999), and heredity (Matkovic et al. 1990, Heaney 1991) influence bone growth, as well as modifiable factors including physical activity (Basey & Ramsdale 1994, Pettersson et al. 2000, Nickols-Richardson et al. (2000), Tsuzuku et al. 2001, Hagberg et al. 2001, Hara et al. 2001, Snow et al. 2001, Faulkner et al. 2003, Greendale et al. 2003), nutrition (Matkovic et al. 1990), environmental behaviours such as cigarette

smoke (Gerdhem and Obrant, 2002), and conditions such as sunlight exposure (Valimaki et al. 2004).

### **2.2.1. Genetic Factors**

Genetics may play a role in the relationship between muscle and bone because muscle and bone share similar growth factors. According to Eisman et al. (1991), genetics is thought to account for 60-80% of the variability in bone growth, while the remaining 20-40% is influenced by modifiable environmental factors. Bone mineral density (BMD), muscle mass and physical fitness all have similar genetic determinants and it is possible that they share a common basis rather than a causal relationship. For example, if an individual is genetically predisposed to growth factors that result in an increase in both muscle and bone, as a result, because of an increase in stature and muscle mass, it would be expected to also perform well on physical fitness tests of strength or power. Also, because of the commonalities between muscle and bone it also is possible that local muscle strength might be a stronger predictor of BMD than physical fitness.

### **2.2.2. Nutrition and bone growth**

Nutritional factors play an important role in bone mass accumulation. Calcium, protein and calcitriol (the active form of vitamin D), for example, provide the building blocks and positive influence necessary for optimal growth and maintenance of strong bones.

There is a positive relationship between calcium intake and bone strength. After reviewing the role of calcium, Heaney (1991) stresses that calcium is not

the cause of bone health but simply a necessary condition for it. In a two-year intervention study by Matkovic et al. (1990) calcium intake and calcium supplementation were analyzed. The authors found the main determinant of calcium balance was dietary intake, while urinary calcium did not change with changes in dietary intake. The group that received a calcium supplement had a greater bone mass over time than the control group. They suggested that inadequate calcium intake may translate into inadequate calcium retention and a reduction in peak bone mass. They also postulated that there could be a threshold of calcium intake that optimizes the effect of calcium uptake on bone.

Several nutritional factors also affect bone growth. In another study by Matkovic et al. (2004) it was noted that the consumption of dairy products had a greater effect on bone than supplementation alone. It was hypothesized that this relationship could be primarily due to the mineral and protein content of dairy products. It was hypothesised by the authors that the protein in dairy products could lead to increased muscle growth and as a result stimulates a subsequent increase in bone. Iwamoto et al. (2004) studied vitamins D and K and calcium intake on bone mineral accrual in rats. They not only found that vitamins D and K positively influenced bone mineral accrual. but that they had an additive with calcium intake on bone mineral accrual. When Vitamins D and K were taken in conjunction with Calcium the effects on bone mineral accrual were above that which would be expected by the addition of the effects of the nutrients. In summary, for optimal bone mineral accrual, a healthy diet with sufficient nutrient

intakes, including calcium, is necessary to provide the appropriate resources for the creation and maintenance of healthy, strong bones.

### **2.2.3. Physical activity and bone growth**

It is well established that an increased level of physical activity has a positive effect on both muscle (Pettersson et al. 2000, Hagberg et al. 2001, Faulkner et al. 2003) and bone (Basey & Ramsdale 1994, Pettersson et al. 2000, Nickols-Richardson et al. 2000, Tsuzuku et al. 2001, Hagberg et al. 2001, Hara et al. 2001, Snow et al. 2001, Faulkner et al. 2003, Greendale et al. 2003, Valimaki et al. 2004). The removal of physical activity also has a negative effect on both muscle (Demiel et al. 1998, MacIntyre et al. 2001) and bone (Demiral et al. 1998, Lehtonen-Veromaa et al. 2001, MacIntyre et al. 2001, Snow et al. 2001, Uusi-Rasi et al. 2001). There is strong support for the positive effects of physical activity on bone mineral accrual. In a study by Moisio et al. (2004), they investigated the association between non-invasive measurements of bone mass and markers of dynamic and static hip bone loads. In subjects expected to be at peak bone mass (31 participants ages 30-49), using DXA at the hip and calculated external joint moments during walking and jogging, they found that external joint moments of the hip explained 40% of the variance in BMD at the hip and 58% of the variance in BMC at the hip. Body mass and height did not increase the explanatory model compared to external joint angles alone. These data support the hypothesis that variance in peak bone mass is associated with dynamic hip loads and is largely independent of the effect of static factors such as height and weight. Thus, it is the local action of physical activity that

stimulates osteogenic activity with physical activity, and not merely an increase in size or mass. Whether it is the result of local muscle action or the loading of bones through impact is not certain.

The method through which physical activity has an effect is likely complex and related to many factors, including genetics, gender, hormones, and lifestyle choices that are often associated with healthy living. When analyzing the contributors to bone mineral accrual, it is difficult to isolate the effects of a single variable, such as the effects of body weight, muscle contraction or ground reaction forces. Typically increases in one of the effects stated above are accompanied by increases in the others. As a result, a cause and effect relationship is very difficult to establish for a mechanism by which physical activity enhances bone mineral accrual.

Physical activity balanced with proper nutrition may have an interactive effect on bone health. Not only does physical activity stimulate osteoblastic activity, but aerobic exercise has also been shown to increase calcium absorption rates. Zitterman et al. (2000, 2002) found that moderate exercise resulted in an acute rise in calcium absorption rates. This effect may be due to an increased response by calcitriol, which is the result of a transient decrease in ionized calcium, which in turn stimulates parathyroid hormone secretion. Individuals who are physically active have increased osteogenic activity as well as an increase in calcium availability for bone accrual.

### **2.3. Muscle and bone**

Tissue mass accumulates and strength increases with normal growth



during childhood and adolescence. Lean tissue mass is related to bone mineral content (BMC) and the relationship is concurrent, meaning the two tissues are expected to show similar changes. Tsuzuku et al. (2001), suggest that bone responds locally to reallocate the forces generated from the muscle at the site of loading. The inefficient lever arms in the body, which are designed for flexible movement and speed and not necessarily maximum force. Therefore, the largest forces applied to the bones result from muscle strain forces acting on the load bearing bones and not from compression forces from body weight, as was previously thought (Frost and Schoenau, 2000). The muscle-bone unit is a result of the forces muscles must overcome due to the resistance of body weight multiplied by the inefficient force producing lever arms against which muscles work. Bones adapt to strains placed on them to ensure that the typical peak strain falls below this strain level. If the stress placed on bone exceeds the typical strain level, bone adapts by increasing bone mass, altering geometry or architecture in order to increase strength. Conversely, if the strain level stays below the remodelling threshold, disuse-mode remodelling removes bone next to the endosteum causing a disuse pattern osteopenia. The range of typical strain levels that falls in between both strain thresholds leads to bone maintenance.

### **2.3.1. Bone and its relationship to muscle**

Several studies have found a relationship between muscle or lean tissue mass and bone mass during adolescence (Wang et al. 1999, Schoenau et al. 2000, Valdimarsson et al. 1999, Taaffe et al. 2001, Heinonen et al. 2001), or muscle strength and bone mass during adolescence (Snow-Harter et al. 1990,

Schoenau et al. 1996, Duppe et al. 1997, Witzke et al. 1999, Petterson et al. 2000, Pettersson et al. 2000). As Seeman (2001) clearly stated, a bigger bone has a bigger muscle. In summary, bone adapts to the specific mechanical loading of the local muscle action.

Faulkner et al. (1993) postulated that bone is a dynamic tissue aligned with the muscular system, and it exhibits changes similar in adolescents to those within the bone of adults. They suggested that because lean tissue is 45-60% muscle, the relationship between lean tissue and bone is most likely due to muscle mass. Changes in muscle mass produce changes in force production (Brooks et al. 2000). Muscle mass can be used as a surrogate measure of muscle strength and force production and is a good predictor of the strain forces acting on bone during muscle contraction.

Athletes have been studied extensively because of the increased physical demands of sport on the body. Bone mineral accrual in gymnasts has been studied widely due to the increased mechanical loading on the body associated with the nature of the sport. Several studies suggest that this is possibly due to increased localized muscle action. Nickols-Richardson et al. (2000) conducted a study on 16 premenarcheal gymnasts along with age, height and weight matched controls. The gymnasts BMD was significantly higher and they also possessed significantly more fat free soft tissue. Nickols-Richardson et al. point out that gymnastics activity may contribute to increases in BMD both directly through impact and loading and indirectly through fat-free soft tissue mass development. In a similar study Faulkner et al. (2003) reported greater

size-corrected BMC at all skeletal sites in premenarcheal elite gymnasts compared to age-matched controls. When BMC was adjusted relative to lean tissue mass, the differences in BMC between the two groups disappeared. Therefore BMC is proportionate to lean tissue mass and with those gymnasts possessing more lean tissue having greater BMC. It is possible that the greater muscle mass resulted in greater mechanical loading and resulting in an increased BMC. However, it was noted that the structural differences between groups could be a result of greater localised bone strains through increased muscular stimulus in the gymnasts.

Wang et al. (1999) attempted to delve further into the relationship surrounding muscle and bone and answer the question as to whether skeletal muscle and bone increase proportionately in growing children. A group of 304 healthy children between the ages of 6 and 18 years were measured for total body potassium (TBK) by  $^{40}\text{K}$  counting and total body calcium (TBC) by dual energy X-ray absorptiometry (DXA). Seventy percent of muscle is potassium and ninety-five percent of bone is composed of calcium; therefore TBK and TBC were used as indices of muscle and bone mass respectively. Wang et al. reported a significant relationship between TBK and TBC. They also observed that boys tend to have a larger increase in the ratio of TBK to TBC than girls and girls have a larger relative increase in TBC to TBK. Therefore boys have more muscle mass than girls, and girls have a larger relative proportion of bone to muscle mass, meaning girls lay down more bone relative to muscle mass than do boys. The authors also found the increase in bone mass was proportionate to the

increases in weight. They also concluded that the growth dynamics of skeletal muscle are weight dependant as well as hormone dependant.

Schoenau et al. (2000) analysed 318 healthy children and adolescents from the DONALD (Dortmund Nutritional and Anthropometric Longitudinal) study between the ages of 6 and 22. They found muscle area accounted for 85% of the variance in cortical area using PQCT (peripheral quantitative computed tomography). Scans were used to measure cortical area and muscle area from a single scan at 65% of the ulnar length. Children of both genders displayed the same relationship, but during puberty males had a greater increase in muscle compared to bone than the females did. BMI did not account for any additional variance. Heinonen et al. (2001) reported significant correlation between muscle cross sectional area with total cortical area in 17 healthy pre-pubertal and early pubertal Caucasian girls between 9-11 years of age. Measurements were completed using MRI (magnetic resonance imaging) as well as PQCT. It was suggested that a larger muscle should be able to elicit a greater mechanical strain on bone; therefore individuals with greater muscle mass should have greater bone mass.

### **2.3.2. Bone and its relationship to strength**

Bone must adapt to the mechanical strain of stronger muscles; therefore, muscle strength should be an indicator of bone strength. Nordstrom et al. (1995) found a site-specific relationship between BMD (bone mineral density) and muscle strength in a study conducted on 28 grade 9 boys using DXA and Biodex. The results indicate a relationship between muscle and BMD that would be the

product of the direct force from muscle action on bone.

Snow-Harter et al. (1990) examined muscle strength and BMD in 59 women 18-31 years of age. Bone mineral was assessed by DXA and muscle strength at the hip, arms, back and legs was measured by 1 repetition maximum test protocol. Results showed significant independent relationships between muscle strength and BMD. Grip strength proved to be the best predictor of mid-radius density, and the strength of hip adductors independently predicted hip BMD. However, they also found muscle strength at locations such as the Biceps to be an independent predictor of lumbar BMD when there is clearly no Biceps muscle attachment. It was suggested that this relationship could be due to stabilization by other muscles during contraction of the muscles completing the desired action from a separate body region or the relationship could be merely correlations between strength indicators.

Valdimarsson et al. (1999) in a cross-sectional study of 254 Icelandic women aged 16, 18 and 20 years aimed at quantifying the inter-relationship between BMD and physical activity, muscle strength and body composition. Using a linear regression model, they found that 58% of the variance of BMC was explained by lean mass, 2.4% by fat, 4.2% by height, 5.5% by years since age of menarche (in females) and only 0.2% by physical activity. Although physical activity has been shown to stimulate bone mineral accrual, in the current study the relationship was masked by that of lean mass. Of the 5 variables tested, only three were significantly related to BMD. Approximately 30% of the variability in total body skeletal BMD was predicted by lean mass and physical activity with

5.9% being explained by years since menarche. A key finding in this study was the significant interrelationship between physical activity, lean mass and grip strength; this finding supports the hypothesis that physical activity plays an important role in affecting peak bone mass.

### **2.3.3 Bone and its relationship to physical activity**

During the growing years, physical activity has been shown to increase bone mass above what could normally be achieved with growth alone (Bailey et al. 1999). Adolescence is a critical period for increased physical activity because during this period the elevated modelling and remodelling activity compounds the effects of physical activity. Together modelling and remodelling create a window of opportunity in which the benefits of physical activity have the greatest potential for increasing bone strength later in life (Bailey et al. 1996). While, physical activity has repeatedly shown a positive effect on bone accrual. the mechanisms are not yet fully understood. Activities that are dynamic and high impact appear to have the greatest effect, probably due to the mechanical stresses that are placed on the bone and the bone accommodation.

Physical activity requires repetitive muscle contractions to produce the desired movement patterns. The muscle-bone unit hypothesis proposes that localised muscle action on bone stimulates an osteogenic response and therefore bone mineral accrual should be related to physical activity. In a study by Delvaux et al. 2001 static arm hang, running speed and upper body endurance were determined to be the best predictors of bone mass in 127 boys from the Leuven Longitudinal Study. The participants were asked to perform a

number of physical activity and motor fitness tests including: leg lifts, sit-ups, bent-arm hang, grip strength, sit and reach and vertical jump. Bone mass was determined by DXA. A physical activity questionnaire and standard grade increasing maximal oxygen consumption test were also completed. Tests that required a great deal of muscular strength, such as running speed and arm hang, best predicted bone mass.

Petterson et al. (2000) looked at the bone mineral differences among late adolescent skippers (n=10, mean age 17.8 years, average 6.1 hours/week skipping), soccer players (n=15, mean age 17.6 years, average 5.1 hours/week training) and controls (n=25, mean age 17.4, average or 0.9 hours of physical activity/week). They found that both skippers and soccer players had higher BMD than controls. Level of physical activity, height, muscle strength and lean body mass were all significant predictors of BMD. The skippers, however, had greater BMD at the tibia. It was suggested that the local muscle action spanning the tibia would be greater while skipping rope compared to playing soccer. Therefore, the greater muscle action in that region could have lead to higher BMD in the associated area. Soderman et al. (2000) also studied adolescent soccer players. They found that when comparing 51 female soccer players (mean age 15 years) with 42 non-active controls (less than 3 hours per week of activity) that muscle strength, as measured by biodex, was positively associated with BMD. In another study, adolescent female cross-country skiers (mean age of 16) had higher BMD at specific sites compared to age-matched controls

(Pettersson et al. 2000). The cross-country skiers' thigh muscle strength was the best predictor for total body, femoral neck, humerus, tibia and femur BMD.

Tsuzuku et al. (2001) studied the effects of high and low intensity weight training on BMD in college aged males (18-25). They found that when compared to controls, weight trained individuals had a higher BMD and that the level of intensity of training was a factor in the amount of bone accrual. This suggests that local muscle action may elicit local osteoblastic activity in bone. Also, there may be a dose relationship between the intensity of physical activity and the response by bone. This result suggests the load placed on bone elicits a proportionate osteoblastic response.

#### **2.3.3.1 Bone and its relationship to physical activity in unilateral sports**

As stated previously, bone mineral accrual is affected by many factors such as nutrition, genetics and hormones. Bilateral studies are thus powerful designs to examine the effects of loading on bone, because the non-dominant limb serves as the control site. Therefore the changes in muscle and bone are activity related through mechanical loading (either through muscular activity or impact loading from the sport). There is a bilateral difference in participants playing sports where one limb is used more than the other. The bilateral effect leads to greater mechanical loading from muscle action in the dominant arm compared to the non-dominant limb. Tennis players have shown greater BMC, endocortical area, and periosteal area in the dominant limb (Nara-Ashizawa et al. 2002). Therefore, tennis players have greater bone mass and better indices of



mechanical strength in their dominant limb, while cortical thickness was the same, thus minimising bone mass.

Haapasalo et al. (2000) studied 12 former Finnish national level players with age, height and weight-matched controls. When measured by PQCT there was a significant side-to-side difference in both groups, although the differences were significantly larger in the tennis players compared to the control group. The additional bone mass in the tennis players was accounted for in bone size (total cross-sectional area, cortical wall thickness), not volumetric density, and these changes seemed to be site specific and the result of mechanical loading at the site.

Daly et al. (2004) recently studied tennis players over the growing years. They looked at 47 competitive female tennis players aged 8-17 years recruited from metropolitan Melbourne, Australia. Using MRI and self-assessed Tanner staging, they found a linear relationship between muscle area and BMC, total, medullary and cortical area at all pubertal stages. However, the intercept for the relationship was higher in post-pubertal tennis players when compared to peri-pubertal and pre-pubertal participants. This could be due to the deceleration and/or cessation of adolescent growth at the later stages of puberty. When comparing playing to non-playing arm, the authors found that the playing arm had greater muscle size, bone size and bending strength than the non-playing arm. Tennis players provide a causal relationship between muscle and bone but an association between the two.

In summary, when looking at athletes who participate in unilateral sports, the dominant limb, which undergoes significantly greater mechanical loading, has greater bone mineral accrual over the non-dominant limb. Since all other factors related to bone mineral accrual are the same for both limbs, it can be deduced that the increases in bone mass must be directly related to increased usage and localised muscle action.

#### **2.3.4. Bone and its relationship to muscle atrophy**

In a study conducted on 41 patients with varying spinal chord injuries from C4 to L3, there was a correlation found between loss of limb function and BMD of the limb (Demiel et al. 1998). Both tetraplegics and paraplegics had equal bone loss in the legs, while tetraplegics lost more bone in the arms. Also patients with a greater degree of spasticity had greater BMD compared to those who were more flaccid. Serum calcium, phosphorous, liver and renal functions were all functioning normally and age and sex had no relationship with bone loss.

Therefore, bone loss was related to the loss of muscle function and the degree to which the local muscles were able to mechanically stimulate osteoblastic activity in bone. If the individuals had control of their limbs they did not lose muscle mass in those limbs and also the degree of muscle tension was a significant predictor of BMD. In a follow-up study conducted on 60-65 year old women Uusi-Rasi (2001) found that decreases in physical fitness, as measured by leg strength, were associated with decreases in BMC. A decreased body weight was also associated with decreased BMC at all measured sites, which would be expected if there were a true relationship. DXA was used for BMC measurements and a

Biodex for measuring leg extension muscle strength. These results suggest that when the ability to place a mechanical load on bone is diminished, there is a subsequent reduction in bone mass.

MacIntyre et al. (2001) showed that muscle recovered after a period of only three months following the removal of a cast, whereas bone took a full year. A total of nine young adults completed the study out of the 16 original participants. Half of the participants wore a cast on their dominant, the other half on their non-dominant hands for a period of 6 weeks. By choosing individuals who had not suffered a break, the effects of trauma or repair of tissue was removed, which allowed the effects of immobilisation on tissue mass to be isolated. DXA scans were completed pre and post casting. An exercise intervention program was implemented with a follow up a year from the removal of the cast. Results showed a loss in BMC at the periosteum, but no decline in BMD. Results showed that the bones cross-sectional area was reduced without changes in bone density; thus the moment of inertia decreased, resulting in a reduction in bone strength. Muscle strength declined but not cross-sectional area; therefore, the measured muscular losses were only functional and not anatomical and were likely from a reduction in neural innervations of the muscle. As a result, the muscle would not be able to place as much force on the bone, even though the muscle mass remained unchanged. This would lead to a reduced strain, which would in turn lead to an increase in bone resorption.

### **2.3.5. Muscle and Bone Summary**

According to the literature, physical activity, lean mass and muscle strength are strongly correlated and significantly related to bone mass and/or bone density. Faulkner et al. (1993) were the first to compare soft tissue analysis to bone in adolescents. Bone free lean tissue was found to be the dominant predictor of total body BMD in both girls and boys when measured by DXA, while weight did not account for any additional variance in the linear regression analysis. They concluded that bone is a dynamic tissue aligned with the muscular system, and it exhibits changes similar to those in muscle. Therefore, the magnitude and timing of one tissue must be aligned with changes in another tissue as the adolescent grows. Therefore, it is plausible that by increasing muscle strength or mass, there would be a subsequent increase in the demands for an increase in bone strength by local skeletal tissue. This increase demand would elicit bone mineral accrual in the corresponding tissue increasing BMC as one of the components of bone strength under normal conditions.

### **2.4. Growth and Maturation**

Growth is a quantitative increase in size or mass. This process continues from the earliest stages of life until death with changes in size, shape or mass (Malina and Bouchard, 2004). Maturation differs from growth in that it constitutes physical changes that occur between conception and maturity and is usually the result of internal processes. Maturity can drastically alter the degree of growth experienced in a given period of time by an individual. Linear growth is a product of the compounded annual growth from birth until the attainment of adult stature.

Growth velocity is the amount of growth that occurs over a given time period. During childhood, growth is fairly constant, until puberty, when the rate of tissue growth accelerates markedly. Velocity of growth accelerates to a peak rate during adolescence and then declines into adulthood.

As the human body grows and matures, form must meet function. This adaptation process is the result of the body's accommodation or adjustment to the immediate environment. Therefore, the growing body should adapt to an increased level of physical activity and mechanical loading by increasing muscle mass, and the increased loading on bone should increase bone accrual. Muscle strength and body weight increase during growth and increases in both height and weight have been associated with increases in BMC and BMD (Slemenda et al. 1994). Additional weight, due to an increase in tissue mass, causes loads on bone to exceed the modeling threshold eliciting a corresponding increase in bone mass and strength to support the additional load. Also, an increase in height is matched with an increase in limb length that requires greater bone strength for support. Bone must adapt at a rate that provides the body with the structural support it requires; therefore, lean mass, muscle strength and BMD are all developed equivalently during puberty (Thorsen et al. 1999).

#### **2.4.1. Gender differences during growth**

Bailey et al. (1999) demonstrated significant main effects for gender and physical activity on peak bone mineral content velocity (PBMCV). Gender is non-modifiable; therefore physical activity may be the most influential modifiable factor affecting bone mineral accrual and an emphasis should be placed on the

promotion of physical activity to achieve an elevated bone mass. Even though females follow the same basic pattern of growth as males, there are differences in the tempo and timing of growth. Females reach peak growth approximately 2 years ahead of males and attain a lesser peak magnitude than their male counterparts (Iuliano-Burns et al. 2001). The reduction in total growing time; compounded by a reduction in peak growth velocities, result in females attaining a shorter stature, and possessing less absolute lean and bone mass. Schoenau (2000) found a strong correlation between muscle area and bone area for both genders. After puberty females have a greater proportionate increase in cortical area relative to muscle area, although absolute values for males are larger. As a result, females have a thicker cortical bone layer compared to their muscle mass than do males. The relative increase in cortical BMC is similar for both genders, but periosteal apposition proceeds longer for males than females (Schoenau et al. 2000, 2002). This extended growth period leads to a larger external bone size for males creating a greater cross-sectional moment of inertia, and therefore a stronger bone structure. Slemenda et al. (1994) found estimated rates of increases in muscle mass to be significantly greater in boys during puberty, but before puberty found similar values in both males and females. It is suspected that sex hormones, mainly estrogens, lead to the gender differences and increases in bone but not muscles in girls. It has been postulated that the relative increase in bone in females compared to males could be a calcium reservoir for gestation and lactation later in life. The principal differences though are that collectively, males have more lean tissue, a greater bone mass and are

taller (Illiano-Burns et al. 2001).

#### **2.4.2. Peak growth velocities**

During adolescence, growth follows a pattern where peak tissue velocities progress in order of: height, lean tissue and finally bone (Lloyd et al. 1998, Iuliano-Burns et al. 2001). Peak height velocity is representative of longitudinal bone growth while PBMCV is most representative of appositional bone growth. According to the literature, it is generally accepted that on average PHV occurs around the age of 12.1 years for girls and 2 years later in boys (14.1 years of age). Peak weight occurs approximately 3 months following peak height and peak strength approximately 1 year after PHV. Peak lean tissue velocity (PLTV) occurs around the same time as height and PBMCV approximately 1 year later (Malina and Bouchard, 1991). In a previous paper from the PBMAS (Pediatric Bone Mineral Accrual Study) Illiano-Burns et al. (2001) found that the age of PHV was 11.8 for females and 13.4 for males. The age of PLTV was 13.7 for males and 12.1 for females. Finally the age of PBMCV velocity was 14.0 for males and 12.5 for females. Thus, males reach peak approximately a year and a half after females, allowing males a longer growing time compared to females. The peak velocities for males were 10.4 cm/year, 8.8 kg/year, and 407 g/year at PHV, PLTV and PBMCV respectively. For females the values were all lower at 8.6 cm/year, 5.2 kg/year and 325 g/year at PHV, PLTV and PBMCV respectively.

#### **2.4.3. Growth of body segments**

For individual body segments, growth accelerates from distal to proximal locations during the pubertal growth spurt. The feet and hands accelerate first

followed by calf and forearm, hips and chest, and finally the trunk (Malina and Bouchard, 2004). Therefore, the more distal regions reach their peak growth velocities prior to the more proximal locations. Maynard et al. (1998) conducted 465 annual DXA scans on 148 healthy children from the Fels Longitudinal Study. Significant gender differences for BMD occurred for the total body at the ages of 16-18, for the arms and legs at the ages of 12-13 years, and at the pelvis at the ages 16-18 years and age 18 for the spine. For BMC the differences occurred for the whole body and legs at the ages of 15-18 years, at the arms and pelvis at the ages 15-18 years and finally ages 16-18 for the spine. Males are expected to eventually be larger than females, as males reach peak growth velocities they pass the female in terms of total growth. This differentiation between the sexes proceeds as the various tissues reach peak growth in males. The differentiation occurs first in the arms and legs, followed by the pelvis and finally the spine.

Bachrach et al. (1999) utilized DXA to measure bone growth at various skeletal locations during a 4-year, mixed-longitudinal design. For both males and females, the hip was the first skeletal area to reach a growth plateau (14.1 years for females and 15.7 years for males) followed by the spine (15.7 years for females and 17.6 for males) and the whole body (16.4 for females and 17.6 for males). These data followed a distal to proximal procession with hip growth plateau occurring before that of the spine. Therefore, it should be expected that increases in muscle mass and BMC would follow a similar progression regardless of whether looking at whole body measurements or individual body segments. The accelerations should be proportionate and occur in a distal to



proximal fashion for the various body segments.

## **2.5. Summary**

Bone and muscle share common hormonal and genetic influences and should, as a result, respond to both growth and physical activity in a similar manner for each gender. Increases in one tissue should be proportionate to increases in the other. Longitudinal data is unavailable to most researchers. Recently using the PBMAS data set, Rauch et al. (2003) found that for mean longitudinal data PLTV preceded PBMCV by 0.57 years in girls and 0.36 in boys. Also PLTV was independently associated with PBMCV ( $r^2=0.50$ ). The legs followed a similar pattern with a separation of 0.30 years in females and 0.11 years in males and the arms by 0.71 years in females and 0.63 years in males. The conclusions from this study were based on data generated from mean or group data. By creating individual growth curves for each individual in the study the participant's individuality and outliers are not lost in the mean. Also, the changes an individual goes through with growth can be observed, rather than a snap shot view of several individuals of different ages and maturities inferring a change that could occur, as is done with cross-sectional studies. This study should be the first to use complete longitudinal data from annual bone and lean tissue mass measurements from various body segments in order to establish a relationship between muscle and bone growth in individuals of various maturational groups and physical activity levels. Lean tissue mass will be used as a surrogate of mechanical forces applied to bone, which would result in a load that should elicit a growth response.

This study will be unique in that it utilises longitudinal data to analyse the relationship between bone free lean tissue and bone in separate body segments. Limbs have no visceral organs and are also the sites of isolated mechanical loading from muscle action. Therefore, the muscle-bone relationship should be most evident in the limbs. Also, the breakdown should allow for analysis of the timing of increases in muscle mass and BMC within separate anatomical sites and allow for the comparison of between them.

## **2.6. Hypotheses**

The literature has given reason to hypothesize that:

### **Hypothesis 1:**

The mean of individual tissue growth in both genders will proceed with increases in peak lean tissue followed by a subsequent increase in peak bone mineral content.

Sub hyp 1: There will be no difference with respect to the timing of peak lean tissue and peak bone mass between the genders when aligned by biological age.

### **Hypothesis 2:**

The progression of peak bone mineral content will proceed from the distal anatomical sites to the more proximal anatomical sites.

Sub hyp 1: There will be no difference in the progression between genders.

### **Hypothesis 3:**

There will be a positive relationship between the magnitudes of bone accretion and lean mass increases for selected anatomical sites.

Sub hyp 1: The ratio of bone to muscle will be greater in females than males.

Sub hyp 2: This magnitude will be more evident in the limbs.

### **3. Methods and Procedures**

#### **3.1 Research design**

This study was an analysis of the age and magnitude of peak growth velocities of children who participated in the University of Saskatchewan Pediatric Bone Mineral Accrual Study (PBMAS). The PBMAS was a prospective mixed-longitudinal analysis design with overlapping cohorts of children. The study was funded for a period of six years plus an additional year, from 1991-1997 with current on-going follow-up studies (2002-2005). Data was collected as participants grew and developed in order to determine how their environment might affect their growth and development. There was no manipulation or control of variables, merely observations allowing for the determination of a relationship between variables and not an attempt to determine a cause and effect relationship by manipulating environmental confounders. The methods for data collection are described in detail elsewhere (Bailey et al. 1999, Bailey, 1997), but are summarized below.

#### **3.2 Participants**

The participants were children in grades three to eight from two Saskatoon elementary schools. Of the original 375 students (ages 8-14) from two Saskatoon elementary schools in 1991, 228 students provided written consent (113 boys, 115 girls). Participants entered the study between 1991 and 1993 and were measured repeatedly in eight age cohorts. By 1993 the original study had recruited a total of 251 participants. At the completion of the original study in

1997, the age range between the youngest initial participant and the oldest final participant was 8-21 years of age. The overlapping cohorts allowed for an estimate of a 13-year developmental pattern from a period of only 7 years. Ninety-four percent of subjects were Caucasian, (Bailey et al. 1999) and from middle socioeconomic neighbourhoods. They were included in a sample that had no history of chronic disease, medication use, or medical conditions that were known to affect growth (Bailey, 1997).

Sufficient longitudinal data was available from 72 boys and 70 girls to fit growth curves and determine the age of peak height velocity (PHV), peak lean tissue velocity (PLTV) and peak bone mineral content velocity (PBMCV).

The original PBMAS study received ethical approval (appendix B) from the University and Hospital Advisory Committee on Ethics in Human Experimentation and was funded by the National Health Research and Development Program of Health Canada (Bailey, 1997).

### **3.3 Anthropometry**

Height and weight were measured for each participant by trained researchers following standard anthropometric techniques outlined by Ross and Marfell-Jones (1991). Participants were required to wear minimal clothing (i.e. shorts and t-shirt) and to remove shoes or jewellery (Faulkner et al. 1996). Stretch-stature height was measured using a wall-mounted stadiometer to the nearest 0.1 cm. Weight was measured on a calibrated electronic scale to the nearest 0.1 kg. (Bailey et al., 1999)

### **3.4 Tissue assessment**

Tissue assessment was performed annually at Saskatoon's Royal University Hospital by a trained technician using a QDR-2000 Hologic Dual Energy X-ray scanner in fan beam mode. Participants were required to remove any metal objects such as jewellery or buttons. The scan times took between 1.5 and 5 minutes depending on the location of the scan. The participants were required to remain as motionless as possible during this period. The procedures for the bone densitometry scans are outlined in the Hologic Quantitative Digital Radiography Operator's manual and user's guide (Hologic 1991). To view a sample DXA scan output see appendix E.

#### **3.4.1 Dual Energy X-ray Absorptiometry (DXA)**

The most common method for assessing bone status in individuals is by DXA. DXA provides a non-invasive and precise procedure for measuring bone mineral content (BMC) and bone mineral density (BMD) (Taaffe et al. 1998). DXA involves the use of a beam of X-ray photons that scans the desired location. There is minimal risk in this procedure with the radiation dose being similar to that one would receive making a return flight from Saskatoon to Halifax on a commercial airline. The total radiation dose per session is less than 10 mrem, while the average annual background radiation in Saskatoon due to natural sources is approximately 150 mrem.

The beam of X-ray photons is composed of both low-energy photons and high-energy photons. The low-energy photons penetrate only the soft tissue surrounding the bone, while the high-energy photons penetrate both the soft

tissue and bone. A detector plate on the opposite side of the tissue location collects and measures the penetrating photons. The collected photons are measured allowing the examiner to express the composition into three tissue types: bone mineral, adipose tissue and lean tissue (Gilsanz, 1999), making DXA a three-compartment model for tissue assessment. The bone mineral is measured as mass of mineral content and expressed as BMC (g) or BMD ( $\text{g}/\text{cm}^2$ ).

Dual energy x-ray absorptiometry (DXA) was originally designed for the purpose of determining body composition in mature adults. DXA techniques and instruments rely on techniques calibrated for adults and measurement constants that were derived by using an adult population (Ellis et al., 1994). It is currently used most commonly as a measurement tool for determining body fat, lean tissue and bone density (i.e. clinical diagnosis and treatment of osteoporosis).

When measuring bone free lean tissue the error in the precision of measurement was less than 3% (Wallace et al., 1995). The precision for measuring soft tissue is diminished because the scan cannot measure the tissue directly above bone and must make an estimate based on the bone periphery. As the scan area becomes smaller there are less data points available to measure and as a result gives a reduced estimate. The precision was 0.5%, 0.7%, 1.1%, and 2.5% for the total body, trunk, legs and arms respectively. Also, the lines of measurement are set to skeletal sites, which are DXA's primary purpose; this reduces its ability to measure soft tissue, as some bone sites do not match up with soft tissue sites. Other similar studies performed in vivo have found a

coefficient of variation that ranges from 0.29% (Nickols-Richardson et al. 2000) to 3.9% (Lehtonen-Veromaa et al. 2001) depending on the location of the scan on the body and the specific instrument used.

In a study by Ellis et al. (1994) DXA was assessed by comparison to the carcass chemical analysis of 16 pigs. Using two separate software programs the pigs were analysed for the three body composition types (BMC, non-bone lean tissue and adipose tissue). All estimates of body weight measures were highly comparable with the chemical analysis were highly correlated ( $r^2 > \text{or} = 0.98$ ). SEEs were 226-271 g for body weight, 3.5-4.3 kg for fat-free mass, and 35.4-36.5 g for BMC. For bone measurements, when compared with the total carcass ash content, the BMC was approximately 25% below the measured values using both software versions. Conversely, the average fat-free compartment was initially overestimated by 968 g, and then underestimated by 892 g. In another study by Taffe et al. (1998) DXA and qualitative ultra-sound (QUS) were compared in a population of healthy young women. For calcaneal measurements, DXA was significant with QUS techniques for the same BMC measurements with a kappa coefficient of 0.41 and an average percentage agreement of 61%. DXA is a low-cost, accessible and easy-to-use instrument that provides precise and accurate measurement of bone mass in adults that are very reproducible (Gilsanz. 1998).

### **3.4.2 Anatomical sites**

The whole body scans were broken down into sections allowing for the analysis of the whole body scan as individual segments. These segments were broken down as follows (see Appendix E for a sample scan output):



## Lower Limbs

The leg region was defined by the oblique femoral line superomedially, by the vertical leg line inferomedially, and laterally by the vertical line of the Upper Limbs

The arm region was defined superiorly by the horizontal shoulder line, medially by the vertical line bisecting the glenoid fossa, and laterally by the border of the global range of interest (ROI).

## Pelvis

The pelvis was defined superiorly by the horizontal line at the iliac crest and laterally and inferiorly by the oblique lines passing through the center of the femoral neck.

## Trunk [Thoracic (T) lumbar (L) spine; ribs]

The T-spine was defined superiorly by the horizontal shoulder line, laterally by the two vertical spine lines, and inferiorly by the short transverse line at T12-L1. The L-spine region was defined superiorly by the horizontal shoulder line at the iliac crest. The ribs were defined superiorly by the shoulder line, laterally by the vertical arm lines, and medially by the vertical spine line.

## 3.5 Age

Age (chronological age) was calculated by subtracting the test date from the participant's birth date and converting the fraction to a decimal value (years + days/365.25). Biological age was determined by subtracting the age at PHV from the age at peak tissue growth.

### **3.6 Determination of tissue velocities**

The determination of tissue velocity (as well as PHV) requires a minimum of two reference points to determine the rate of change over that period (tissue mass<sub>2</sub> – tissue mass<sub>1</sub>, in g. / time<sub>2</sub> – time<sub>1</sub>, in y.). The calculated velocity was then ascribed to the midpoint between the two ages or the age center. Peak tissue velocity was defined as the maximum rate of tissue gains (g/y) of either lean tissue or bone mineral content. The age of peak tissue velocity was defined as the age at which maximal tissue growth occurs.

### **3.7 Growth Curves**

Growth velocities were derived from growth distance data for height, lean tissue mass and BMC for each individual. A cubic spline was fitted to growth velocities for each individual using the GraphPad Prism software version 4.00 package for all body segments (arms, legs and trunk). A cubic spline procedure provides a smoothed curve that allows for the maximum velocity to be determined over the growth period. Once the cubic spline fits were applied estimates for age and magnitudes at peak tissue growths were determined for both lean tissue and bone mineral content for the three selected regions of interest. Therefore, the peak ages used for statistical analyses were extrapolated along with peak tissue velocities from the cubic spline allowing for a more accurate evaluation of peak for each individual. This method has been used previously (Bailey et al. 2000, Iuliano-Burns et al. 2001). The curves were then used to derive age and magnitude at peak, while participants who have not yet reached or passed peak were separated from the study cohort. To view sample

growth curves for the three selected regions of interest view Appendix F.

### **3.8 Statistical Analysis**

For all statistical procedures an alpha level was set at  $p < 0.05$ . All analyses were completed using SPSS for windows version 11.5. The statistical procedures used to address each of the research questions are outlined below.

#### **3.8.1 Statistical Analysis for Hypothesis 1**

A factorial ANOVA (age x gender) was completed for all three locations of interest. Differences between the age of PLTV and PBMCV were determined by the presence of a significant main effect for age at all three locations.

Differences in the age of peak between boys and girls for all three locations were determined by a significant main effect for gender. A significant interaction (age x gender) would verify if there was a disparity between boys and girls in the progression of growth. A factorial ANOVA (age x gender) was then performed using data aligned by PHV (age PLTV – age PHV & age PBMCV – age PHV) to determine whether gender has a main effect, verifying that when aligned biologically there is no gender difference between participants.

#### **3.8.2 Statistical Analysis for Hypothesis 2**

A factorial ANOVA (location x gender) was completed for the age of PBMCV at all three locations of interest. Differences between the ages of PBMCV between all three locations were determined by the presence of a significant main effect for location. When a significant main effect was found for location, a post-hoc comparison was performed for location to delineate where the significant differences occurred. Differences in the age of PBMCV between

boys and girls for all three locations were determined by a significant main effect for gender. A significant interaction (location x gender) would verify if there was a disparity between boys and girls in the progression of growth.

### **3.8.3 Statistical Analysis for Hypothesis 3**

A forward linear regression model was then completed for PBMCV at all three locations of interest. PLTV was first introduced to the equation as a predictor for PBMCV followed by gender, height at PHV (to control for size) and age at PHV (to control for maturation) to determine which factors were significant predictors of PBMCV at all three locations.

No statistical analyses were made between locations for the forward linear regression model, differences were only noted.

## 4. Results

### 4.1 Participant characteristics

The sample population has been compared previously to values of other adolescent populations, to determine whether the PBMAS sample was a representative sample of average adolescents (Mundt, 2004). These comparisons revealed that male and female participants' height and weight were similar to those who participated in the NHANES study, a CAPHER study, SGDS, and the Canadian Fitness Survey and are represented graphically below (figures 4.1 to 4.4).

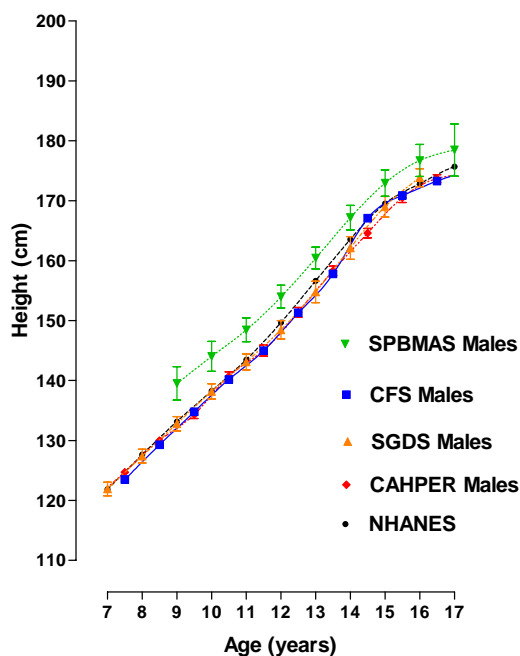


Figure 4.1 – Mean height,  $\pm$  95% CI, of PBMAS males compared to those of four other studies. (Mundt, 2004)

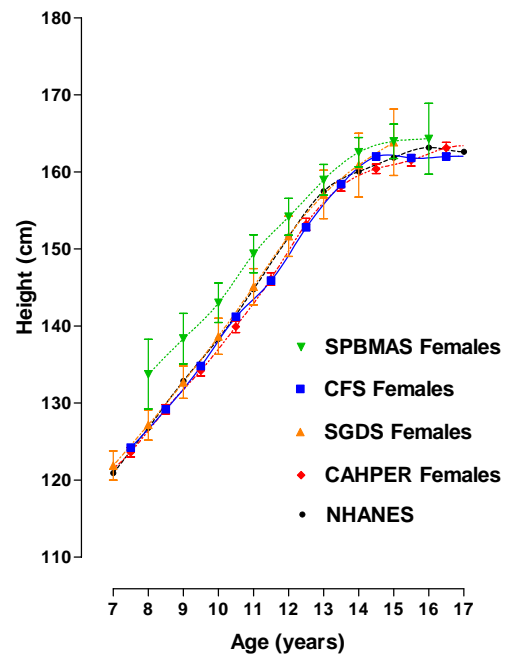


Figure 4.2 – Mean height,  $\pm$  95% CI, of PBMAS females compared to those of four other studies. (Mundt, 2004)

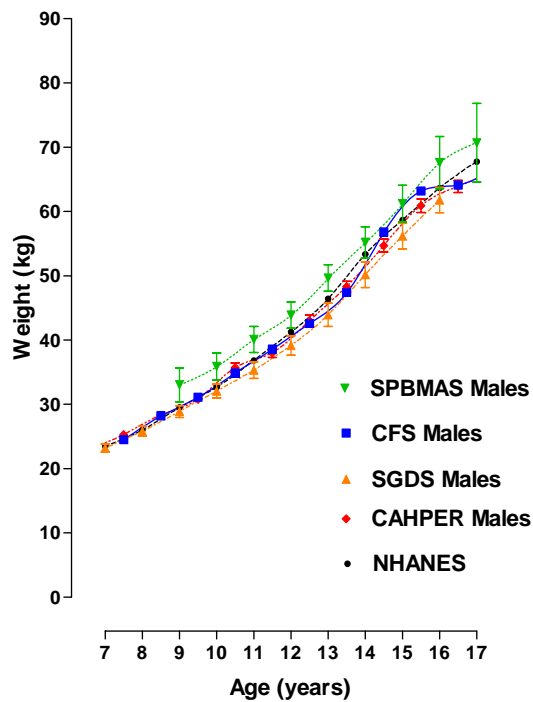


Figure 4.3 – Mean weight,  $\pm$  95% CI, of PBMAS males compared to those of four other studies. (Mundt, 2004)

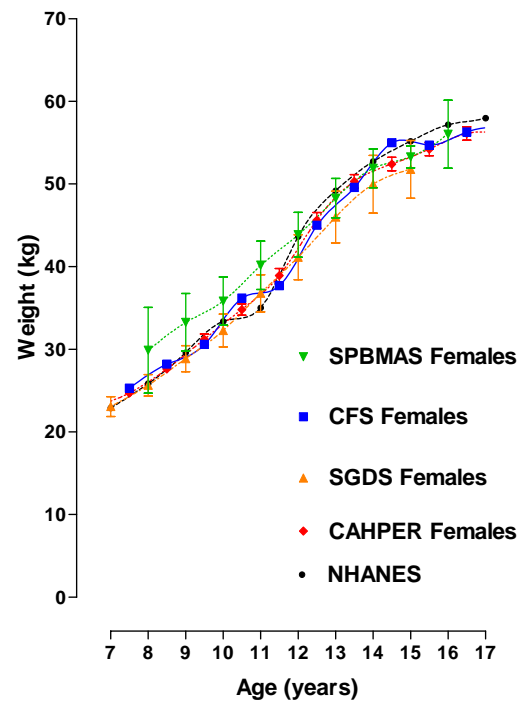


Figure 4.4 – Mean weight,  $\pm$  95% CI, of PBMAS females compared to those of four other studies. (Mundt, 2004)

Of the 72 boys whose data were fitted with growth curves, there was a distinguishable peak for the arms in 44 boys, for the legs in 41 boys, and for the trunk in 43 boys. Of the 70 girls whose data were fitted with growth curves, there was a distinguishable peak for the arms in 44 girls, for the legs in 37 girls, and for the trunk in 42 girls. A complete summary of study population, and magnitudes at peak velocity are provided in Table 4.1.

Table 4.1 Summary of study population, and magnitudes at peak velocity

		Mean(SD) magnitude at peak velocity	
	<u>n</u>	<u>PLTV</u>	<u>PBMCV</u>
<b>Boys</b>			
Arms	44	1171.2 (339.5)	67.0 (17.1)
Legs	41	3358.1 (644.0)	188.9 (40.9)
trunk	43	4827.0 (865.7)	150.5 (31.8)
<b>Girls</b>			
arms	44	527.1 (140.0)	41.75(10.2)
Legs	37	1935.0 (561.7)	141.9 (30.9)
trunk	42	2928.2 (641.9)	113.2 (27.9)

## 4.2 Age of Peak tissue growth

The mean age of PLTV and PBMCV are presented in Table 4.2 for all three sites of measurement. A 2 X 2 factorial ANOVA (age at peak tissue growth x gender) was completed to test for differences between the age of attainment of PLTV and the age of attainment of PBMCV. For both genders there was a significant difference between the age at PLTV and PBMCV at the trunk ( $F(1,170)=4.97$ ,  $P<0.05$ ), and the arms ( $F(1,176)=7.18$ ,  $P<0.05$ ). For boys PLTV significantly ( $P<0.05$ ) preceded PBMCV by an average of 0.28 (SD 0.63) years at the trunk, 0.42 (SD 0.82) years at the arms. There was no significant ( $P<0.05$ ) difference between the age at PLTV and PBMCV at the legs, which had PLTV preceding PBMCV by 0.31 (SD 0.58) years. For girls the age of PLTV significantly ( $P<0.05$ ) preceded the age at PBMCV by an average of 0.44 (SD 0.53) years at the trunk, 0.65 (SD 0.84) years at the arms. There was a difference of 0.24 (SD 0.60) years at the legs without significance ( $P>0.05$ ). There was a significant ( $P<0.05$ ) gender difference for the age at PLTV and

PBMVC between boys and girls at the trunk ( $F(1,170)=74.74$ ,  $P<0.001$ ), the arms ( $F(1,176)=31.21$ ,  $P<0.001$ ) and the legs ( $F(1,156)=64.79$ ,  $P<0.001$ ) with girls reaching peak at an earlier age than boys. However there was not a significant ( $P>0.05$ ) age x gender interaction at any of the three measurement sites. Therefore, even though girls reach peak at an earlier age than boys, there is not a significant difference in the pattern of timing of PLTV and PBMVC between boys and girls. (Appendix C.1 to C3. for further details)

Table 4.2 - Mean ( $\pm$  SD) age at peak tissue growth.

Age at peak velocity		
	<u>PLTV</u>	<u>PBMVC</u>
<b>Boys</b>		
Arms	13.55 (1.26) <sup>a, b</sup>	13.98 (0.22) <sup>a, b</sup>
Legs	13.46 (0.99) <sup>b</sup>	13.78 (1.16) <sup>b</sup>
Trunk	13.67 (1.04) <sup>a, b</sup>	13.95 (1.04) <sup>a, b</sup>
<b>Girls</b>		
Arms	12.32 (1.27) <sup>a, b</sup>	12.97 (1.31) <sup>a, b</sup>
Legs	12.13 (1.05) <sup>b</sup>	12.38 (1.01) <sup>b</sup>
Trunk	12.19 (0.99) <sup>a, b</sup>	12.63 (1.15) <sup>a, b</sup>

<sup>a</sup> significant difference ( $p<0.05$ ) between the age at peak PLTV and PBMVC.

<sup>b</sup> significant difference ( $p<0.05$ ) between genders at selected regions of interest

When participants were aligned by biological age using PHV as the marker (Figure 4.5) there was a significant difference between biological age of PLTV and PBMVC at the arms, legs and trunk with no significant gender by age interaction (Appendix C.4 to C.6 for further details). There was a significant main effect of gender at both the arms ( $F(1,176)= 5.82$ ,  $P<0.05$ ) and trunk ( $F(1,170)=4.85$ ,  $P<0.05$ ) with no difference between genders at the legs. There was a significant difference between the age from PHV for PLTV and PBMVC at the trunk ( $F(1,170)=11.04$ ,  $P<0.001$ ), the arms ( $F=9.81(1,176)$ ,  $P<0.05$ ), and the



legs ( $F(1,156)=6.51$ ,  $P<0.05$ ).

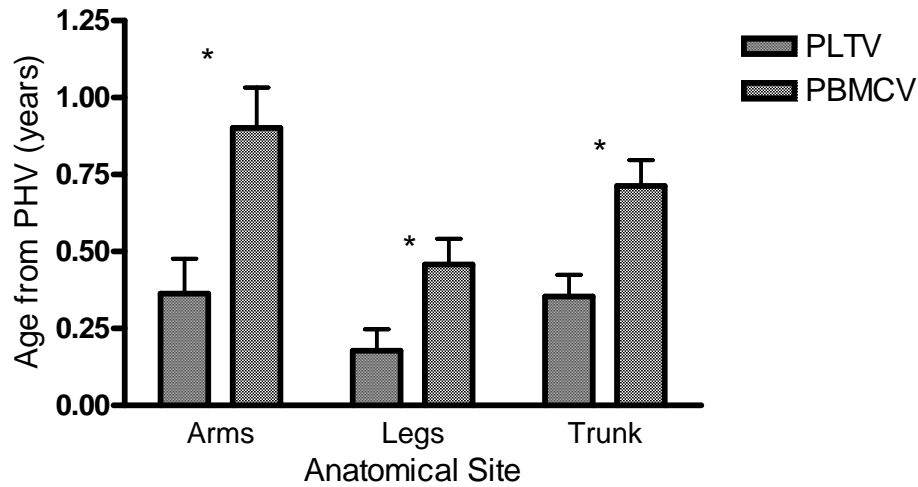


Figure 4.5 Biological age (mean  $\pm$  SEM) of peak lean tissue velocity and peak bone mineral content velocity showing PLTV occurring significantly earlier than the age of PBMCV - \* =  $p<0.05$

Peak lean tissue growth occurred before peak bone tissue growth occurred in 25 (58%), 32 (70%), 27 (66%) of the boys at the locations of trunk, arms and legs, respectively. The number of girls following the pattern was 32 (76%), 33 (75%), 23 (62%) in the locations of the trunk, arms and legs, respectively.

### 4.3 Age of peak tissue growth by location

A Factorial ANOVA (age at PBMCV by location) was completed to determine if there was a significant difference between the age at PBMCV at the arms, legs and trunk. There was no significant difference ( $F(2,251)= 2.213$ ,  $P>0.5$ ) between any of the locations (for full ANOVA Table see Appendix C.7). However, when aligned by PHV a significant difference ( $F(2,251)=4.56$ ,  $P<0.05$ )

in the timing of PBMCV for the selected regions of interest was found (Appendix C.8). A post hoc test (Tukey HSD) revealed that these differences (Appendix C.9) are between the arms and the legs ( $P>0.05$ ) with the peak in bone growth in the legs occurring significantly before peak bone growth in the arms (Figure 4.6).

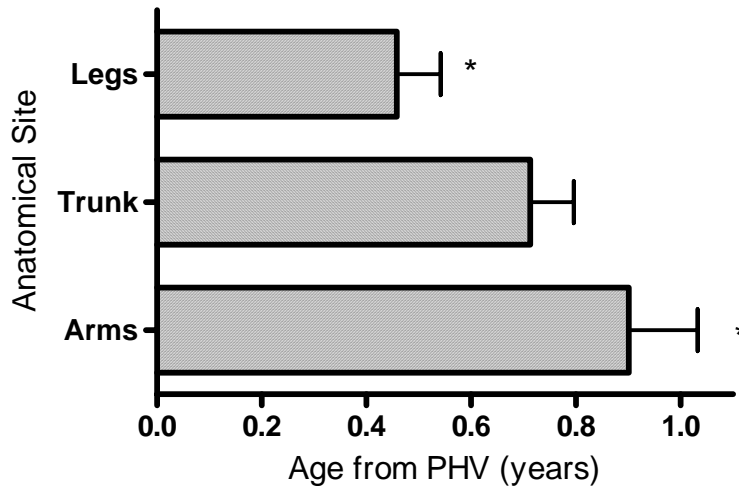


Figure 4.6 Age of PBMCV (mean +/- SD) by anatomical site showing a significant difference between the age of PBMCV at the legs and the arms - \* =  $p<0.05$

#### 4.4 The relationship between PLTV and PBMCV

A forward linear regression model was used to test hypothesis that PLTV, with peak height (PH), gender, and age of PHV as predictors of PBMCV. From the linear regression model, only PLTV was independently associated with PBMCV at the arms ( $r^2 = .71$ ,  $p<0.001$ ), legs ( $r^2 = .53$ ,  $p<0.001$ ) and trunk ( $r^2 = .52$ ,  $p<0.001$ ) (appendix D.1 to D.3). Gender, age of peak height velocity, and height at peak height velocity were not significant predictors of PBMCV and as a result were excluded from the model. The data for the arms, the legs and the trunk are represented in Figures 4.2, 4.3 and 4.4 respectively. The strongest relationship

between PLTV and PBMCV was at the arms ( $r^2 = .71$ ,  $p < 0.001$ ), with the legs and trunk showing a similar, but weaker relationship ( $r^2 = .53$ ,  $p < 0.001$  and  $r^2 = .52$ ,  $p < 0.001$  respectively). Also, the legs and arms had the largest B coefficient, or slope of the regression line (0.038 and 0.34,  $P < 0.001$ , respectively), approximately 50% greater than the trunk.

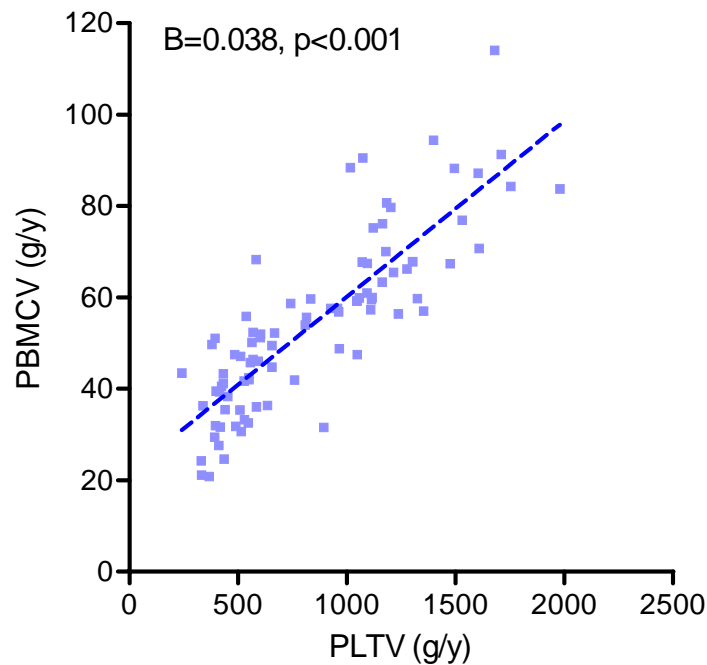


Figure 4.7 - Relationship between peak bone mineral content velocity and peak lean tissue velocity in boys and girls at the arms.

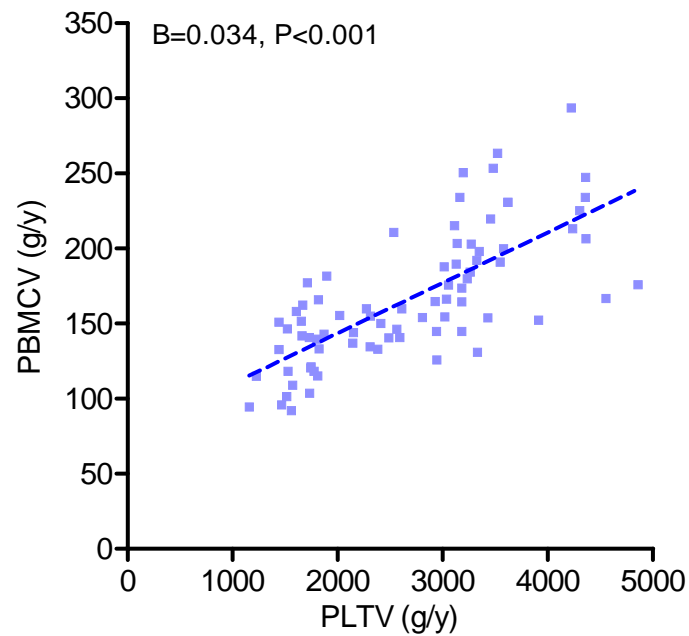


Figure 4.8 - Relationship between peak bone mineral content velocity and peak lean tissue velocity in boys and girls at the legs.

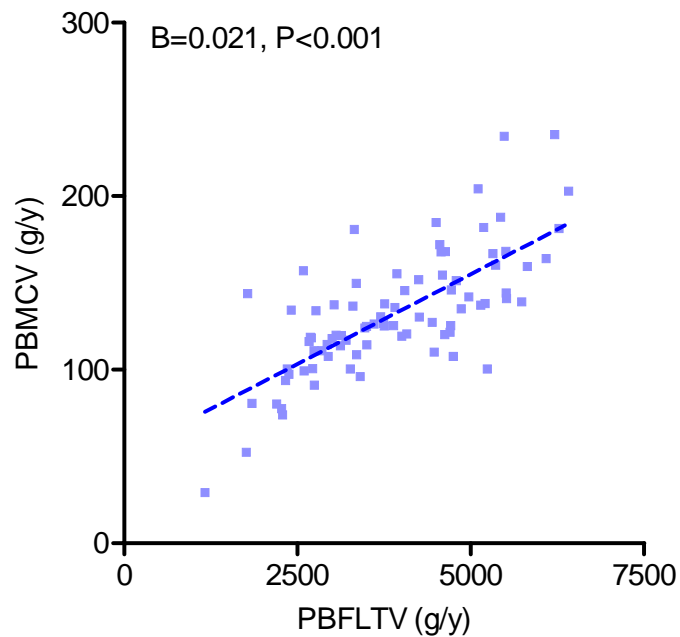


Figure 4.9 - Relationship between peak bone mineral content velocity and peak lean tissue velocity in boys and girls at the trunk.

## **5. Discussion and Conclusion**

### **5.1 Discussion**

#### **5.1.1 General discussion**

The purpose of this study was to explore the timing and tempo of peak growth velocities of lean tissue (LT) and bone mineral content (BMC) in boys and girls at specified sites of the arms, legs, and trunk. It was hypothesized that PLTV would precede PBMCV at each site with girls peaking prior to boys, but there would be no gender difference in the timing when controlling for maturation. It was also hypothesised that growth would proceed from distal sites to proximal sites in both genders. Finally, it was hypothesized that the magnitude of the velocity for lean tissue at peak would be associated with the magnitude of the velocity at the arms, legs and trunk, with there being a gender difference in the ratio of lean tissue growth to BMC accrual.

Unique strengths of the study were the use of longitudinal data to explore the relationship between muscle and bone and the investigation of these relationships at the 3 distinct sites. Longitudinal design made it possible to determine the timing and magnitude of PLTV and PBMCV for each individual. It also made it possible to exclude those individuals who did not attain peak tissue growth during the study. In contrast, cross-sectional studies or pre-post design studies only provide a snapshot and provide only an indicative status of the growth process. Growth curves derived from cross-sectional studies tend to be smooth and mask the wide range of individual variation (Malina et al. 2004). Because cross-sectional studies provide only indicative status, they do not

provide sufficient reference data to allow for the isolation of peak growth. In terms of growth and development where changes occur over long periods of time, longitudinal analyses provide a broad view of each participant that allows for a better understanding of the research question (Malina et al. 2004). Specifically, with regard to the current research design, a longitudinal analysis allowed for the isolation of peak tissue velocity for each individual and a reference age in time. Analyzing the legs, trunk and arms separately was based on the theory that the relationship of the timing of PLTV to PBMCV may be more apparent at the limbs (as compared to the trunk) where muscle actions and/or impact forces may be more isolated on the specific bone site. Muscle accounts for almost the entire lean tissue at the limbs, thus reducing the visceral organs as a confounding variable. In addition, the high muscle content of lean tissue at the limbs should be directly related to mechanical loading on bone in the given region. This study was also unique in that it analysed growth at the legs, arms and trunk separately. This allowed for the relationship between muscle and bone to be explored at different locations having distinct functions.

The results, taken collectively, supported the premise that lean tissue had a significant relationship to bone (BMC) with respect to both timing and tempo of growth and peak velocities at different locations in the body. As hypothesised, the peak growth velocity for lean tissue occurred significantly earlier than the peak growth velocity for bone. Also, there was a significant positive relationship between the magnitude of peak lean tissue growth and peak bone growth. This supports the theory that the muscle-bone unit which proposes that lean tissue is

a driving force for bone mineral accrual (Frost and Schoenau, 2000). The hypothesis that lean tissue and BMC accrual would occur first at the extremities followed by the trunk was not supported. There was a difference between genders in the age of peak for both lean tissue and bone tissue at all locations with female's peak growth occurring before that of males. When genders aligned by maturity (age of peak velocity) the gender differences between the timing of PLTV and PBMCV were removed. With respect to the association between the magnitude of peak lean tissue growth and peak bone mineral content accrual, there were no gender differences found for the ratio of lean tissue to bone mineral growth.

#### **5.1.2 The timing of PBMCV and PLTV (Hypothesis 1)**

The association between muscle forces on bone has been referred to as the muscle-bone unit (Frost and Schoenau, 2000). Based on this theory, it was hypothesised that the action of muscle forces on bone drives bone development then this relationship should be most evident during peak growth when the magnitude of development of tissues is maximised. As shown in the results, PLTV occurred before PBMCV. There was a gender difference with female's peak growth velocities occurring, on average, at an earlier chronological age than males; this result is in accordance with previous research from the same data set (Iuliano-Burns et al. 2001) and other research involving the timing of PBMCV and PLTV in adolescence (Lloyd et al. 1998). There was no gender interaction in the order of the timing of peak growth velocities in the upper limbs and trunk. A significant difference between PLTV and PBMCV in the lower limbs was not

found. When all participants were aligned biologically by PHV, PLTV occurred before PBMCV at all locations in the body as well with no gender differences (Table 4.2). Greater impact loading in the lower body might alter the timing of lean and bone in the legs, causing an advanced osteogenic response in the legs and reducing the time lapse between the two tissues. Also, muscle mass precedes muscle strength (Rasmussen et al. 1990); therefore, bone adaptation may be more dependant on muscular strength at the arms, where the impact forces are reduced compared to the legs.

Recently, using the PBMAS data set, Rauch et al. (2003) found that for mean longitudinal data PLBMV preceded PBMCV in the legs with a separation of 0.30 years in females and 0.11 years in males and the arms by 0.71 years in females and 0.63 years in males. The conclusions from this study were based on data generated from mean or group data. In contrast to Rauch et al., results in this study were based on individual growth data. In the current study PLBMV preceded PBMCV in the legs with a separation of 0.24 (SD 0.60) years in females and 0.31 (SD 0.58) years in males and the arms by 0.65 (SD 0.84) years in females and 0.42 (SD 0.82) years in males for the same data set. The current study used individual peaks rather than the mean peak, and as a result should produce a more accurate description of the growth patterns, although the results are very similar. As discussed previously by Malina et al. 2004, using mean data results in a regression towards the mean masking individual variation and a blunting of the true growth peaks. By extrapolating the peak for each participant individually a more accurate description of the sample population, utilising



individual variation is possible.

### **5.1.3 The timing of PBMCV for individual body segments (Hypothesis 2)**

For individual body segments, theory suggests that growth accelerates from distal regions to more proximal regions in the body during the pubertal growth spurt (Malina and Bouchard, 1991). That is, the feet and hands accelerate first followed by calf and forearm, hips and chest, and finally the trunk. Therefore, it was hypothesised that the distal regions would reach their peak growth velocities for BMC prior to the more proximal locations. The results however did not support this hypothesis. The only significant difference was found between the arms and legs, with PBMCV occurring in the legs earlier than the arms (Figure 4.1). Bass et al. cross-sectionally analysed bone growth for the total body, spine, humerus, radius, femur and tibia in 109 girls (age 6.5-17). They found that appendicular growth was in advance of axial growth and that before puberty growth in the legs was more rapid than growth of the spine. Forty percent of bone mass at the legs was accrued by age 11, whereas only twenty-four percent had been accrued at the spine. Unfortunately there have not been sufficient studies looking at the differences on the timing of peak gains in bone at the arms compared to other sites throughout the body.

The theory that growth proceeds distally to proximally provides a description of the genetic script for human growth. According to Eisman et al. (1991), genetics is thought to account for 60-80% of the variability in bone growth while the remaining 20-40% is influenced by modifiable environmental factors. According to the mechanostat theory, bone growth is stimulated when

microstrains reach the overuse window and stimulates an osteogenic response (Frost. 1989). The arms and legs share many common environmental factors, although there is a large difference in terms of loading from impact forces. Intuitively, in humans, daily activities involving the upper limbs require muscle action, but rarely involve ground reaction forces involved in weight-bearing (MacIntyre et al. 2001). The legs could be in advance of the arms as a consequence of impact forces advancing bone accrual; whereas, at the arms BMC accrual may be more affected by specific muscle actions to stimulate an osteogenic response after PMSV (peak muscle strength velocity). PMSV occurs after peak muscle mass velocity (Rasmussen et al. 1989). Therefore, the combination of ground reaction forces from walking compounded by increases in muscle strength could augment the elicited growth response altering the timing of peak growth at the arms and legs. This would also indicate that the environmental factors affecting growth in the present study would be strong enough to mask the genetic control over growth changes. These findings are in agreement with research by Ruff (2003) who found that body size was the most important element in the weight-bearing lower limb skeleton, while both body size and muscle strength are important in the upper limb, especially in males. Infancy peaks in the velocity of bone growth were reported during the second year of life for the lower-limbs that correspond to the beginning of walking. It was proposed that these results argue strongly for the importance of mechanical factors in the development and shaping of the pre-adult skeleton.

PBMCV at the trunk occurred between the legs and arms; but this difference was not significant, possibly because growth of the visceral organs is independent of either muscular stimulation or impact forces to stimulate growth. The trunk has stabiliser muscles, but the portion of skeletal muscle mass is reduced as a result of visceral tissue. Unfortunately the observational nature of the current study did not allow for the control of impact forces or other factors required in attempting in establishing a cause and effect relationship.

#### **5.1.4 The relationship between the magnitude of PBMCV and PLTV**

##### **(Hypothesis 3)**

As shown in the results, there was a positive linear relationship between PLTV and PBMCV during adolescence. These observations are in accordance with the proposal that local action from muscle forces drives bone accrual as described by the mechanostat theory (Frost and Schoenau, 2000). The results are consistent with other research that examined the relationship between lean tissue mass and bone mineral content measured by DXA during adolescents. Markou et al. (2004) et al found a significant positive relationship between lean body mass and BMD in both males and females between the ages of 13-23 when analysed cross-sectionally. Witzke and Snow (1999) also found a significant correlation between bone free lean mass and BMC in healthy girls with a mean age of 14.6 years. Valdimarsson et al. (1999) found lean mass to be the dominant predictor (58%) of BMC in 358 Icelandic girls of 16, 18, or 20 years of age. Therefore, according to the results of the current study and supportive literature, it can be reasoned that increases in lean tissue mass should be

followed by a subsequent increase in bone mineral content. There is strong evidence that there is a clear association between increase in muscle strength and bone mass (Burr, 1997).

Height at peak height velocity, age at peak height velocity and gender were entered into the model to control for size, maturity and gender influences on the magnitude of growth. None of these variables were significant predictors in the model and as a result were excluded. This is in agreement with previous research suggesting that static factors such as height and weight are not predictors of bone mineral accrual (Moisio et al. 2004). Meaning the movement of physical activity is what stimulates osteogenic activity with physical activity, and not an increase in size or mass. These variables have been shown previously to influence growth, however, in the current model their effects are masked by PLTV's effect on PBMCV. Early maturers have shown to have a greater PLTV, as well as PBMCV (Iuliano-burns et al. 2001); therefore these results would suggest that even though they have an increased peak magnitude, the ratio between the magnitudes remains unchanged. The results also suggest the same holds true for size and for gender. Even though larger individuals typically have a greater magnitude of growth, the ratio remains unchanged.

Gender was also not a significant predictor in the regression analyses. Previous researchers have reported females to have a greater proportionate increase in cortical bone area relative to muscle area, although absolute values for males are larger (Schoenau et al., 2000, 2002, Slemenda et al. 1994). These previous studies however were done on post-pubertal subjects; thus it is possible

that there may be different relationships between genders that are not seen until post-puberty. Daly et al. (2004) found a linear relationship in female tennis players (ages 8-17 years) between muscle area and BMC, total, medullary and cortical area. However they also noted that the intercepts were higher in post- compared to peri- and pre-pubertal groups. These results suggest that independent of age, a female who is more mature had more bone (mass and cortical area) for a given muscle area in post-puberty compared to pre- and peri-pubertal individuals. The changes in cortical thickness might be a result of changes that occur throughout puberty and have not yet occurred at the time of PBMCV. Wang et al. (1999) analysed TBC and TBK children and adolescents ages 6-18 years and proposed that the relationship between skeletal muscle mass and bone mass to be the same in boys compared to adults, but in girls found that the relationship changes with growth and maturation. Therefore it is possible that the gender differences are not a result of peak growth velocities; rather slow changes that occur throughout the growth process as an individual matures into adulthood.

Gender differences were also found in studies measuring cross-sectional area of bone, not bone mass. Schoenau et al. (2000) found that between the ages of 6-7 years and adulthood the relative increase in BMCcort (cortical bone mineral content) was similar between genders. However, the relative increases in polar moment of inertia, section modulus and strength strain indicators were higher in males. The authors also found that the ratios between architectural parameters and BMCcort in post-pubertal males were higher compared to post-

pubertal females. This suggests that for a given mass, males have stronger bones than their female counterparts. Therefore, changes observed between genders in terms of bone strength may perhaps be geometric in nature and not related to bone mass.

It is noteworthy that the arms had the strongest relationship of lean to bone, and then legs closely followed by the trunk. Taaffe et al. (2001) also found that lean mass was the dominant predictor of BMD in the upper limbs. The arms are isolated to local muscle action and most likely are more or less independent of ground reaction forces. These results suggest that local muscle action and ground reaction forces have an independent effect on bone accrual.

It was noted that the increases in PLTV were marked by a greater subsequent increase in PBMCV at the arms and legs than the trunk as indicated by a larger B (legs 0.34, arms 0.38, and trunk 0.21,  $p < 0.001$ ) coefficient, or slope of the regression line. The trunk's visceral organs would remove some of the relationship because they are not theoretically related to the bone mineral accrual through the muscle-bone unit, while in the limbs, where lean tissue is predominantly composed of muscle mass, increases in lean tissue would have a greater influence over bone mineral accrual. Thus, this supports that increases in lean tissue mass (specifically skeletal muscle mass) are followed by a subsequent proportionate increase in BMC, and is more evident at the limbs than the trunk.

Physical activity level was not entered into the regression model in the present study. It has already been mentioned and is well established that an

increased level of physical activity has a positive effect on bone (Basey & Ramsdale 1994, Nickols-Richardson et al. 2000, Snow et al. 2001, Valimaki et al. 2004). There is also evidence of a dose-response relationship, with increased duration (Greendale et al. 2003) of physical activity as well as an increase in intensity-level (Hara et al. 2001, Tsuzuku et al. 2001) producing an increase in BMD. Other studies found comparable results when looking at lean and bone simultaneously (Pettersson et al. 2000, Hagberg et al. 2001, Faulkner et al. 2003). Therefore, with increased levels of physical activity there would be an increase in both muscle and bone similar to with size, maturity and gender. However, if future studies could distinguish between types of physical activity, whether it is high or low impact and whether it led to muscle hypertrophy and/or bone mineral accrual that would be beneficial. This would help determine if high impact activities alone lead to bone mineral accrual or if any activity that leads to muscle hypertrophy leads to a corresponding increase in bone mineral accrual.

According to Daly et al. (2004): other factors associated with growth (or bone accrual) other than muscle size have an adaptive growth response; thus, one unifying hypothesis that larger muscles lead to a proportionate increase in bone mass, size and strength are simplistic and denies the influence of other factors in the development of bone. In other words a model proposes that the growth of lean tissue is the only factor that drives bone growth is too basic and does not take into account other factors that also likely drive bone growth.

## 5.2 Conclusions

Pertaining to the previously stated hypotheses, several conclusions can be made about the current study's results:

1. The mean peak of individual peak tissue growth velocities proceeds with increases in peak lean tissue velocity occurring significantly before peak bone mineral content velocity at the arms, and trunk with no gender interaction (as hypothesised).
  - a. There was no gender difference when subjects were aligned by biological maturity (as hypothesised).
2. Peak bone mineral content velocity occurred significantly earlier in the legs than the arms when aligned by peak height velocity (did not support the hypothesis).
  - a. There was no interaction between gender and the timing of peak bone mineral content velocity (as hypothesised).
3. There was a significant positive relationship between the magnitudes of peak bone mineral content accretion and peak lean tissue accretion at all selected regions of interest (as hypothesised).
  - a. There was no gender difference in the relationship between the magnitudes of peak bone mineral content accretion and peak lean tissue accretion at all selected regions of interest (did not support the hypothesis).



- b. The relationship between the magnitudes of peak tissue growth was stronger at the arms (as hypothesised).
- c. Increases in peak lean tissue velocity marked a greater increase in PBMCV at the arms and legs compared to the trunk (as hypothesised).

In conclusion, the findings of this study support the Muscle-bone Unit (Frost and Schoenau, 2000), which theorises that localised muscle action is a driving force for bone accrual. There is a relationship between the timing and the tempo of muscle and bone growth during the adolescent growth spurt with increases in lean mass preceding a consequent increase in bone mass.

#### **5.4 Limitations**

It must be noted that none of the above findings prove a direct cause and effect relationship. It is likely that there are a large number of determinants that affect bone growth during adolescence and that local muscle action is component of the forces that elicit osteogenic responses in the growing skeletal system. Also the findings are specific to adolescent populations that were recruited for the study. This relationship between muscle and bone is greater in adolescents because modelling is inactive in adults (Burr, 1997).

Limitations of the present study are the use of surrogates for both bone strength and muscle strength. BMC is only a component of bone strength; a true measure of bone strength would also need to take into account bone geometry and architecture. Also, a direct measurement of muscle strength and the corresponding forces elicited on the body would be beneficial in future research.

Also mathematical equations were used to derive an estimate for peak age and magnitudes for whole year measurements. Whenever possible, especially during the growing years, more frequent measurements allow for a more accurate description of growth curves. Finally, it must also be remembered that DXA is an indirect measurement of body composition. Therefore there is error associated with the measurement that must be taken into account. This error may also become more evident in longitudinal studies where a change over time is expected or when looking at ratios between tissues (Ellis et al. 1994). Also when the scans were completed, even though the land-marking for anatomical sections was completed by a trained technician, some variation is expected due to the nature of the methodology.

#### **5.4 Future directions**

Further studies are needed pertaining to the timing and magnitude of bone mineral accrual as it relates to muscle growth. If possible studies should be longitudinal in nature covering enough time to capture the changes that occur with growth while controlling for maturity. Future studies need to analyse bone strength as it relates to local muscle strength and usage while controlling for confounding variables. Further studies should also explore the relationship at different stages of life, more specifically the aging senior population where osteoporosis is of importance. If local muscle action can elicit osteogenic response, individuals who are at risk of osteoporotic fracture can be prescribed activities that stimulate bone mineral accrual while minimising the risk of injury,

such as swimming or aqua size. These activities would improve bone mass without requiring high impact loading and increasing the risk of fracture.

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**APPENDIX A**

**Sample Consent Form**

Parent's statement:

I understand the purpose and procedures of this study as described above and I voluntarily agree to allow my child to participate. I understand that at any time during the study, he or she will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of the consent form, the proposed procedures and possible risks.

I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study.

\_\_\_\_\_  
Signature of Parent or Guardian

\_\_\_\_\_  
Date

Subject's Statement:

I understand the purpose and procedures of this study as described above and I voluntarily agree to participate. I understand that at any time during the study, I will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I understand the contents of the consent form, the proposed procedures and possible risks.

I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study.

\_\_\_\_\_  
Signature of Subject

\_\_\_\_\_  
Date

Skeletal fragility in older adults appears to be a function of peak bone mass attained in early adult years. Nutritional factors and mechanical loading factors during the growing years may have an impact on the attainment of an optimal level of bone mass, this is the rationale behind the current study on children.

Heredity is another factor that may be involved in osteoporosis. For this reason provision will be made for any mother, who is interested, to have her own bone density status evaluated. This will provide each participating mother with the most accurate indication of bone density status currently available, and when linked with the data on their child it will provide us with valuable generational information.

As the mother of a child in the bone density study, I am interested ☐ I am not interested ☐  
in having my own bone mineral content and skeletal status evaluated at some time during the next year.

Please return this form to Mr. Kikcio, Principal, Prince Philip School by Wednesday April 17, 1991. If you were unable to attend the parent information meetings and have questions about the proposed study, Mrs. Barb Mooney at Alvin Buckwold School (374-0811) can provide you with additional information or you can contact Dr. Don Bailey, the chief investigator for this study, at the University of Saskatchewan (966-6524).

## **APPENDIX B**

### **Copy of Ethics Approval**



UNIVERSITY ADVISORY COMMITTEE ON ETHICS IN  
HUMAN EXPERIMENTATION

(Medical Ethics)

NAME AND EC #: Dr. D.A. Bailey  
Physical Education

88-102  
NHRDP (#6608-1261-OS)

DATE: February 15, 1994

The revised consent form for the study entitled "A mixed Longitudinal Study of Bone Density Change During the Adolescent Years in Boys and Girls with Special Reference to Physical Activity Patterns and Nutritional Factors" has been reviewed by the University Advisory Committee on Ethics in Human Experimentation (Medical Sciences) and approval has been provided for renewal of the study.

Therefore you are free to proceed with the project subject to the following conditions:

APPROVED.

Please submit the revisions requested in 1(a) to the Director of Research Services, Room 210 Kirk Hall.

2. Any significant changes to your protocol should be reported to the Director of Research Services for Committee consideration in advance of its implementation.
3. Please submit to the Committee all adverse events reports received from the study sponsor.
4. Upon discontinuation or closure of the research study, please notify the Director of Research Services in writing.

  
E. A. McKenna  
Chair  
University Advisory Committee on Ethics in Human Experimentation

cc: Royal University Hospital

**APPENDIX C**  
**ANOVA Tables**



**Table C.1** - Factorial ANOVA between age of peak tissue growth and gender at the trunk.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	88.687(a)	3	29.562	26.646	.000
Intercept	29203.023	1	29203.023	26321.665	.000
GENDER	82.918	1	82.918	74.737	.000
TISSUE	5.518	1	5.518	4.974	.027
GENDER * TISSUE	.279	1	.279	.252	.617
Error	184.172	166	1.109		
Total	29516.555	170			
Corrected Total	272.859	169			

a R Squared = .325 (Adjusted R Squared = .313)

b location = trunk

**Table C.2** – Factorial ANOVA between age of peak tissue growth and gender at the arms.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68.626(a)	3	22.875	12.902	.000
Intercept	30677.329	1	30677.329	17301.893	.000
GENDER	55.328	1	55.328	31.205	.000
TISSUE	12.723	1	12.723	7.176	.008
GENDER * TISSUE	.575	1	.575	.324	.570
Error	304.967	172	1.773		
Total	31050.921	176			
Corrected Total	373.592	175			

a R Squared = .184 (Adjusted R Squared = .169)

b location = arms

**Table C.3** - Factorial ANOVA between age of peak tissue growth and gender at the legs.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	75.493(a)	3	25.164	22.522	.000
Intercept	26040.723	1	26040.723	23306.944	.000
GENDER	72.384	1	72.384	64.785	.000
TISSUE	3.009	1	3.009	2.693	.103
GENDER * TISSUE	.051	1	.051	.046	.831
Error	169.829	152	1.117		
Total	26496.083	156			
Corrected Total	245.322	155			

a R Squared = .308 (Adjusted R Squared = .294)

b location = legs

**Table C.4** - Factorial ANOVA between age from PHV of peak tissue growth and gender at the trunk.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.193(a)	3	2.731	5.462	.001
Intercept	48.543	1	48.543	97.092	.000
GENDER	2.423	1	2.423	4.847	.029
TISSUE	5.518	1	5.518	11.037	.001
GENDER * TISSUE	.279	1	.279	.559	.456
Error	82.995	166	.500		
Total	139.483	170			
Corrected Total	91.188	169			

a R Squared = .090 (Adjusted R Squared = .073)

b location = trunk

**Table C.5** - Factorial ANOVA between age from PHV of peak tissue growth and gender at the arms.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20.853(a)	3	6.951	5.357	.001
Intercept	70.347	1	70.347	54.213	.000
GENDER	7.549	1	7.549	5.818	.017
TISSUE	12.728	1	12.728	9.809	.002
GENDER * TISSUE	.576	1	.576	.444	.506
Error	223.187	172	1.298		
Total	314.387	176			
Corrected Total	244.040	175			

a R Squared = .085 (Adjusted R Squared = .069)

b location = arms

**Table C.6** - Factorial ANOVA between age from PHV of peak tissue growth and gender at the legs.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.927(a)	3	1.309	2.832	.040
Intercept	16.099	1	16.099	34.833	.000
GENDER	.818	1	.818	1.771	.185
TISSUE	3.009	1	3.009	6.511	.012
GENDER * TISSUE	.051	1	.051	.111	.740
Error	70.250	152	.462		
Total	89.948	156			
Corrected Total	74.177	155			

a R Squared = .053 (Adjusted R Squared = .034)

b location = legs

**Table C.7** - Factorial ANOVA between age of PBMCV by location and gender.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	102.577(a)	5	20.515	14.083	.000
Intercept	44097.924	1	44097.924	30272.586	.000
GENDER	96.311	1	96.311	66.116	.000
LOCATION	6.446	2	3.223	2.213	.112
GENDER * LOCATION	1.814	2	.907	.623	.537
Error	356.890	245	1.457		
Total	44859.389	251			
Corrected Total	459.467	250			

a R Squared = .223 (Adjusted R Squared = .207)

**Table C.8** - Factorial ANOVA between age from PHV of PBMCV by location and gender.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.696(a)	5	3.339	3.797	.002
Intercept	119.822	1	119.822	136.256	.000
GENDER	6.367	1	6.367	7.240	.008
LOCATION	8.031	2	4.016	4.566	.011
GENDER * LOCATION	1.822	2	.911	1.036	.357
Error	215.449	245	.879		
Total	354.995	251			
Corrected Total	232.145	250			

a R Squared = .072 (Adjusted R Squared = .053)

**Table C.9** – Post Hoc test of the Factorial ANOVA between age from PHV of PBMCV by location and gender. (Tukey HSD)

(I) location	(J) location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
trunk	arms	-.1884	.14261	.385	-.5247	.1479
	legs	.2548	.14704	.195	-.0920	.6015
arms	trunk	.1884	.14261	.385	-.1479	.5247
	legs	.4432(*)	.14583	.007	.0993	.7871
legs	trunk	-.2548	.14704	.195	-.6015	.0920
	arms	-.4432(*)	.14583	.007	-.7871	-.0993

Based on observed means.

\* The mean difference is significant at the .05 level.

## **APPENDIX D**

### **Linear Regression Analyses**

Table D.1 - Regression Model Summary for arms

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.843(a)	.711	.708	10.21621

a Predictors: (Constant), magnitude of peak lean tissue velocity arms

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	21.710	2.496		8.700	.000
	magnitude of peak lean tissue velocity arms	.038	.003	.843	14.556	.000

a Dependent Variable: magnitude peak bone mineral content velocity arms

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	gender	-.034(a)	-.369	.713	-.040	.388
	age of peak height velocity	.006(a)	.094	.925	.010	.813
	height at peak height velocity	.134(a)	1.910	.060	.203	.658

a Predictors in the Model: (Constant), magnitude of peak lean tissue velocity arms

b Dependent Variable: magnitude peak bone mineral content velocity arms

**Table D.2 - Regression Model Summary for legs**

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.726(a)	.527	.521	29.93510

a Predictors: (Constant), magnitude of peak lean tissue velocity legs

**Coefficients(a)**

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	76.484	10.358		7.384	.000
	magnitude of peak lean tissue velocity legs	.034	.004	.726	9.207	.000

a Dependent Variable: magnitude peak bone mineral content velocity legs

**Excluded Variables(b)**

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics Tolerance
1	gender	.023(a)	.183	.856	.021	.415
	age of peak height velocity	-.077(a)	-.907	.367	-.104	.864
	height at peak height velocity	.129(a)	1.153	.253	.132	.491

a Predictors in the Model: (Constant), magnitude of peak lean tissue velocity legs

b Dependent Variable: magnitude peak bone mineral content velocity legs

**Table D.3** - Model Summary for trunk

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.717(a)	.515	.509	24.65193

a Predictors: (Constant), magnitude of peak lean tissue velocity trunk

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	51.642	8.982		5.749	.000
	magnitude of peak lean tissue velocity trunk	.021	.002	.717	9.383	.000

a Dependent Variable: magnitude peak bone mineral content velocity trunk

Excluded Variables(b)

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics Tolerance
1	gender	.073(a)	.594	.554	.065	.387
	age of peak height velocity	-.105(a)	-1.273	.207	-.139	.857
	height at peak height velocity	.150(a)	1.477	.143	.161	.557

a Predictors in the Model: (Constant), magnitude of peak lean tissue velocity trunk

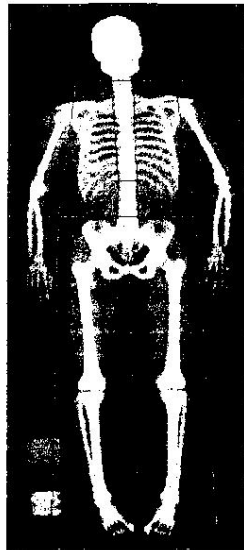
b Dependent Variable: magnitude peak bone mineral content velocity trunk

## **APPENDIX E**

### **Sample DXA Scan Output**



# Nuclear Medicine Dept - RUH - Saskatoon



·Sep 19 11:32 2003 [333 x 152]  
Hologic QDR-2000 (S/N 2124)  
Enhanced Array Whole Body U5.73A

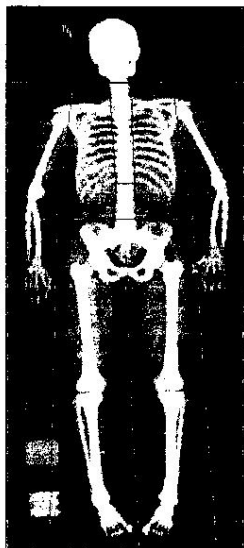
A0919030E Fri Sep 19 11:10 2003  
Name: Helgason, Nial  
Comment: Cross Compare 123  
I.D.: CC-007 Sex: M  
S.S.#: 111-11-1111 Ethnic:  
ZIPCode: Height: 185.00 cm  
Scan Code: BW Weight: 87.00 kg  
BirthDate: 05/23/80 Age: 23  
Physician: WLKNSN,DUDZC  
Image not for diagnostic use

TOTAL BMC and BMD CV is < 1.0%  
C.F. 1.004 1.045 1.000

Region	Area (cm2)	BMC (grams)	BMD (gms/cm2)
L Arm	260.33	225.37	0.866
R Arm	263.12	246.44	0.937
L Ribs	149.73	126.04	0.842
R Ribs	132.56	111.03	0.838
L Spine	184.06	197.28	1.072
L Spine	64.68	85.89	1.328
Pelvis	331.00	474.05	1.432
L Leg	405.12	607.55	1.417
R Leg	403.12	658.59	1.363
SubTot	2353.71	2812.25	1.195
Head	200.69	614.97	2.191
TOTAL	2634.40	3427.22	1.301

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# Nuclear Medicine Dept - RUH - Saskatoon



·Sep 19 11:32 2003 [333 x 152]  
Hologic QDR-2000 (S/N 2124)  
Enhanced Array Whole Body U5.73A

A0919030E Fri Sep 19 11:10 2003  
Name: Helgason, Nial  
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S.S.#: 111-11-1111 Ethnic:  
ZIPCode: Height: 185.00 cm  
Scan Code: BW Weight: 87.00 kg  
BirthDate: 05/23/80 Age: 23  
Physician: WLKNSN,DUDZC  
Image not for diagnostic use

?  
F.S. 68.00% 0(10.00)%  
Head assumes 17.0% brain fat  
LBM 73.2% water

Region	Fat (grams)	Lean+BMC (grams)	% Fat (%)
L Arm	644.0	4344.4	12.9
R Arm	537.6	4716.3	10.2
Trunk	3028.8	34707.1	8.0
L Leg	2741.3	12543.7	17.8
R Leg	2928.2	12612.1	18.8
SubTot	9079.8	69023.7	12.5
Head	1007.3	4612.5	17.9
TOTAL	10887.2	73636.3	12.9

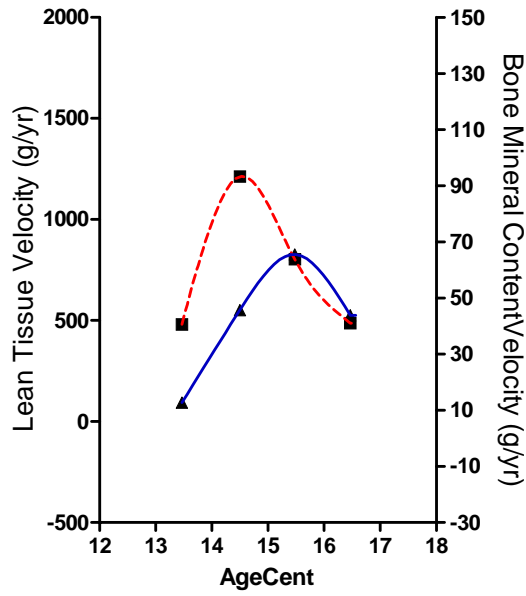
HOLOGIC

## **APPENDIX F**

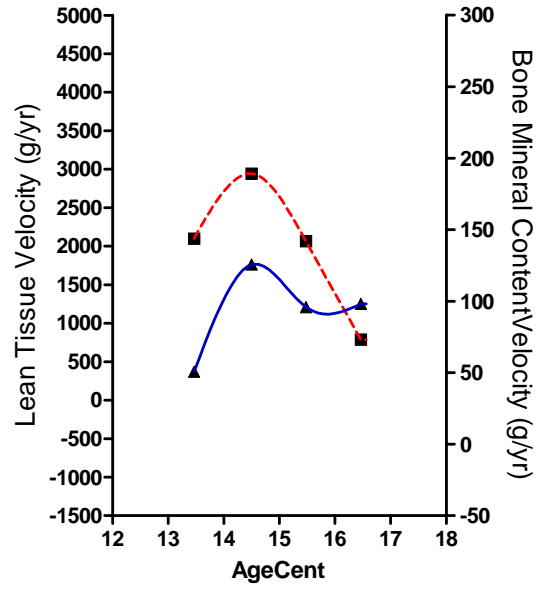
### **Sample Growth Velocity Curves**

**Figure F.1 Sample Growth Velocities for PLTV and PBMCV**

a. growth velocities for PLTV and PBMCV at the arms



b. Growth velocities for PLTV and PBMCV at the legs



c. Growth velocities for PLTV and PBMCV at the trunk

